CHEMISTRY OF NATURAL PRODUCTS

(SUMMARY)

Thesis
Presented to The Aligarh Muslim University
For
The Degree of Doctor of Philosophy
In
Chemistry

By
SYED AZIZ AHMAD

1973

This is to certify that the thesis is the original work of the candidate and is suitable for submission.

Asif Zaman

(Asif Zaman)
Supervisor.

SUMMARY

The thesis describes the results of studies undertaken on the constituents of five Indian medicinal plants, Calliscarpa macrophylla, Calliscarpa lanatifolia, Pluchea microcarpa, Rhamnus triquetra and Colebrookia oppositifolia. The major part of the thesis is devoted to the structure of two new diterpenes which were isolated from Calliscarpa macrophylla and designated initially as CM-1 and CM-2. These names had to be revised subsequently for reasons given below:

Structural studies showed CM-1 to be the acetate of CM-2 and established a tetracyclic carbon skeleton for the parent alcohol. These conclusions are based chiefly on the NMR and IR spectra of the two compounds which, besides establishing the nature of the carbon skeleton, provided proof of the presence of a primary and a tertiary hydroxyl and a carbonyl function in a kaurane or a phyllocladane nucleus. The primary alcoholic, CH_2OH protons appear as doublets in the alcohol and singlet in the acetate spectrum which suggested that one of the hydroxyls is attached to C-17. Since this could not be regarded as conclusive efforts were directed towards finding a chemical proof for the location of the primary and tertiary hydroxyl groups as well as the carbonyl function. It was hoped that reduction of the carbonyl, either by the Huang

Minlem procedure or by Raney nickel desulpherisation of the derived thioketol, would deliver a compound identical with a known phyllocladane or kaurane diol. After some difficulties, connected with the separation of the products formed in the Huang Minlem reduction, a compound agreeing in melting point with phyllocladane 16,17-diol was obtained. The identity was confirmed by its conversion to the corresponding monoacetate and oxidation to 17-nor-phyllocladane-16-one, the melting points of which also agreed with those of the corresponding phyllocladane derivatives. The identifications were finally confirmed by comparison with authentic samples of these compounds kindly undertaken by Prof.R.C.Cambie.

It now remained to establish the position of the carbonyl function. While this work was in progress, A.Chatterjee *et al* reported isolation of two compounds calliterpenone and calliterpenone-monoacetate from *Callicarpa macrophylla* the melting points of which agreed with CM-2 and CM-1 respectively. The identity of CM-2 with calliterpenone was confirmed by comparison with an authentic sample of calliterpenone provided by Prof.Chatterjee. In order to avoid confusion in literature the designations CM-2 and CM-1 were dropped in favour of calliterpenone and calliterpenone monoacetate. These authors, however, assigned a kaurane nucleus to calliterpenone which is not in accord with the results reported here. They carried out reduction and dehydration studies which appeared to show that the carbonyl is present

at C-11 and the compound was therefore given the 11-oxo-kaurane-16,17-diol structure. The results of similar studies carried out during the course of this work were not conclusive and an alternate procedure was relied upon for establishing the position of this carbonyl. This consisted of conversion of calliterpenone acetonide to a mono bromide and observing the splitting pattern of the C$\underline{H}$Br proton. The NMR spectrum of the bromide showed this proton as a quartet which eliminated the 11-oxo-phyllocladane structure. Only two positions for the carbonyl, C-1 and C-3, are compatible with this type of splitting of the proton under bromine. Peracid oxidation of calliterpenone acetonide gave a product in which two of the methyls were deshielded which can only happen if the carbonyl in the original compound is placed at C-3. Calliterpenone was thus conclusively shown to be 3-oxo-phyllocladane-16,17-diol.

The discussion of the structure of calliterpenone is preceeded by a brief review of methods employed in structure elucidation of tetracyclic diterpenes. The chemistry of tetracyclic diterpenes, their biogenesis and NMR spectral characteristics are also discussed in this connection.

Of the other plants investigated none afforded an unknown compound. However, the substitution pattern of the flavones isolated from Calebrochia serratifolia, which were reported to exist in

Calliearpa japonica in a paper which appeared while this work was in progress, is still somewhat unusual. Similarly, though a known compound bergenin from *Ficusaga microcarpa* is also a comparatively rare plant constituent. The quinones from *Rhamnus frignatra* are on the other hand among the most common quinonoid pigments of natural occurrence. Investigation of *Calliearpa longifolia* revealed that calliterpenone is here not accompanied by its acetate.

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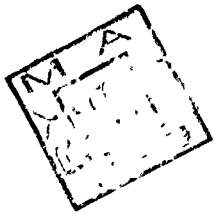

A C K N O W L E D G E M E N T

The research work recorded in this thesis was carried out under the guidance of Dr.Asif Zaman. I am greatly indebted to him for his interest and encouragement throughout this work.

I also wish to thank Prof. W.Rahman, Head, Department of Chemistry, for providing research facilities.

I express my appreciation and thanks to Dr.Abu Shoeb, C.D.R.I. Lucknow for recording UV, IR and NMR spectra and Prof.J.Reisch, Institut fur Pharmazeutische Chemie, Munster, West Germany, for providing some NMR spectra.

Thanks are also due to my colleagues for cooperation and helpful discussions.

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Syed Aziz Ahmad

Dated July 28, 1973. (Syed Aziz Ahmad)

CONTENTS

INTRODUCTION

INTRODUCTION

A number of medicinal plants were collected personally from the Dehra Dun region and their chemical constituents were studied. This thesis is based on the work on five such plants from which compounds belonging to defferent classes of natural products e.g. terpenoids, coumarins and flavonoids were isolated and identified.

This work offered an opportunity of gaining experience in spectroscopic methods of characterisation of compounds belonging to diverse structural types. Specially useful from this view point was the work on the diterpenoids constituents of Callicarpa macrophylla.

Elucidation of the structure of the diterpene alcohol isolated from this plant required its conversion to a known derivative, phyllocladane-16,17-diol. Various methods available for the reduction of the carbonyl function were explored in this connection.

Application of certain other reaction was necessary to establish unambiguously the position of the carbonyl function. This work made it possible to get acquainted with modern methods of structural elucidation.

From the other plants only known compounds, the isocoumarin bergenin, emodin, frangulin, and some flavones were obtained but a study of the NMR and mass spectra was also of considerable value.

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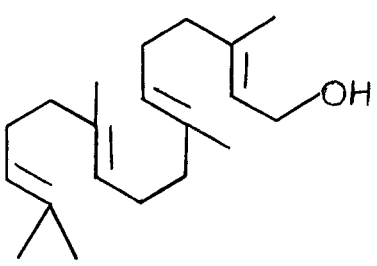
THEORETICAL

The tetracyclic diterpenes form a very important group of naturally occurring diterpenoids. A survey of the literature of this class of compounds revealed that upto the middle of 1972 a total of 500 diterpenes, exclusive of alkaloids, were isolated. Out of this very substantial number 105 belong to the tetracyclic type, represented by kaurenes 80, atisanes 22 and phyllocladanes 3. Apart from their importance due to this numberical strength tetracyclic diterpenes gain added significance from the position they occupy in the biological evolution of cyclic diterpenoids from the acyclic precursor, geranylgeraniol (I) or its biochemical equivalent. Thus the intermediacy of a cation such as (II) in the biogenesis of tetracyclic diterpenes was suggested by Wenkert (1) and was apparently justified by the conversion of rimuene (III) to isophyllocladene (IV) in refluxing formic acid. This lent a special significance in diterpene chemistry to rimuene the structure of which remained uncertain till it was finally settled by Ireland's synthesis (2,3) of all the four possible pimaradienes (V-VIII), with which it was considered identical at one time or other, and finally of rimuene (III) itself (4,4a,4b) in 1966. A further point of interest in the chemistry of these compounds is the importance acquired by gibberellic acid (IX) as a plant growth hormone (5). Apart from this gibberellic acid is the diterpene (so classified even though it has only 19-carbon atoms because of its biogenetic relationship to kaurene) the biogenesis (6) of which has been most thoroughly studied using labelled precursors. It belongs to the group of compounds (7) derived by oxidative modifications and rearrangements of kaurene (X).



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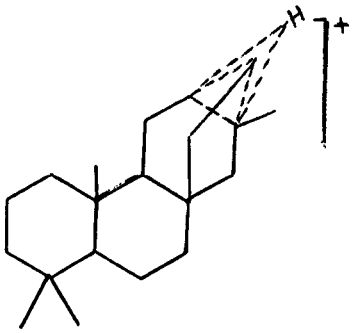

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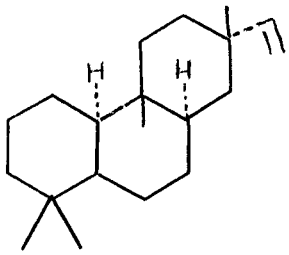

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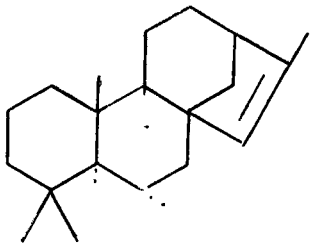

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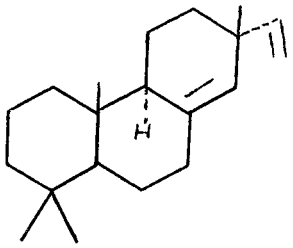

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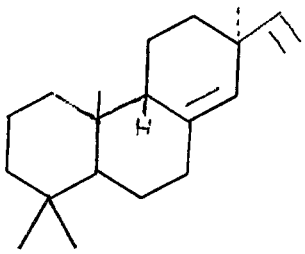

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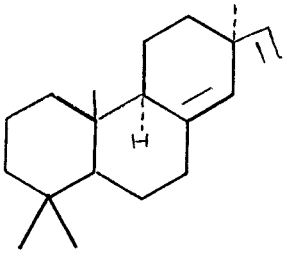

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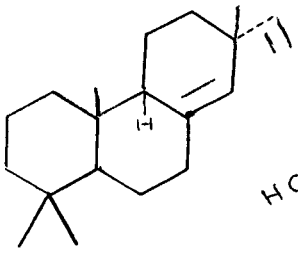

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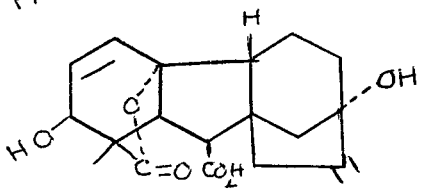

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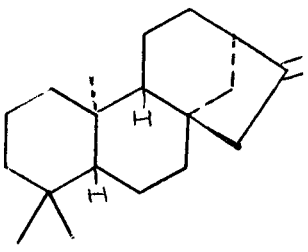
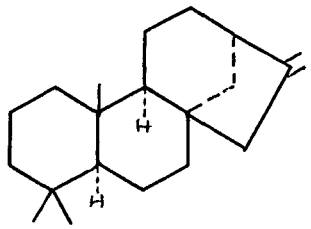


X

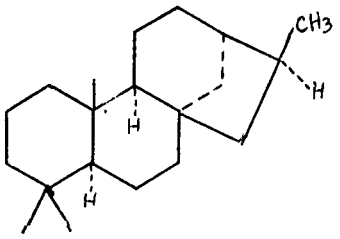
Much of the chemistry of tetracyclic diterpenes was studied in connection with structural investigations on phyllocladene. The structure proposed initially by Brandt (8) was supported by subsequent studies of Grant and Hodges (9,10) who also established the stereochemistry of the A B ring junction. This work and that of Briggs, Cambie and their co-workers (11,12) led to the proposal of the absolute stereochemistry shown in (XI) for phyllocladene. The main features of this work are summarised below:



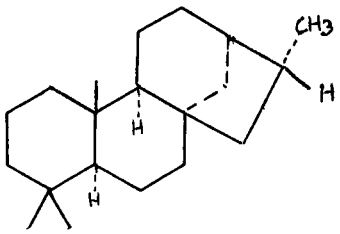
(XI)

Brandt isolated phyllocladene from Araucaria angustifolia and noted that it was isomerised to a hydrocarbon isophyllocladene by treatment with H_2SO_4 through a process which must involve protonation of the double bond followed by proton elimination from the β-position, since hydrogenation of both phyllocladene and isophyllocladene gave the same mixture of dihydro derivatives, later designated as phyllocladane (XII) and epiphyllocladane (XIII). The position of the gemdimethyl groups and the angular methyl was assumed on the basis of analogy with other diterpenes. Dehydrogenation to retene (7-isopropyl-1-methyl phenanthrene) provided evidence

for the site of fusion of the 5-membered ring.

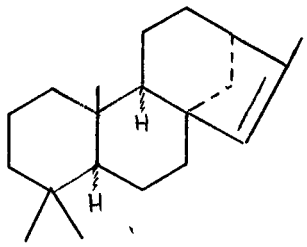


(XII)

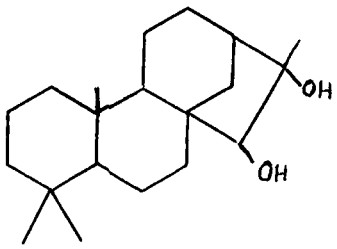


(XIII)

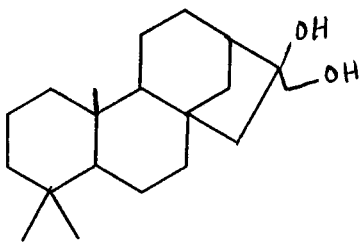
Acetone permanganate oxidation of phyllocladene in presence of water afforded a glycol $C_{20}H_{34}O_2$, a ketone $C_{19}H_{30}O$ and an exoacid $C_{19}H_{30}O_3$. Oxidation of isophyllocladene afforded along with a diol an exoacid $C_{20}H_{32}O_3$ which gave a positive iodoform reaction indicating the presence of an acyl group in the molecule the location of which on C-13 of the tricyclic diterpene carbon skeleton follows from the formation of 1-methyl-7-ethylphenanthrene on selenium dehydrogenation of its methyl ester. These results were accommodated by Brandt in the structural proposals (XI) and (XIV) for phyllocladene and isophyllocladene respectively.



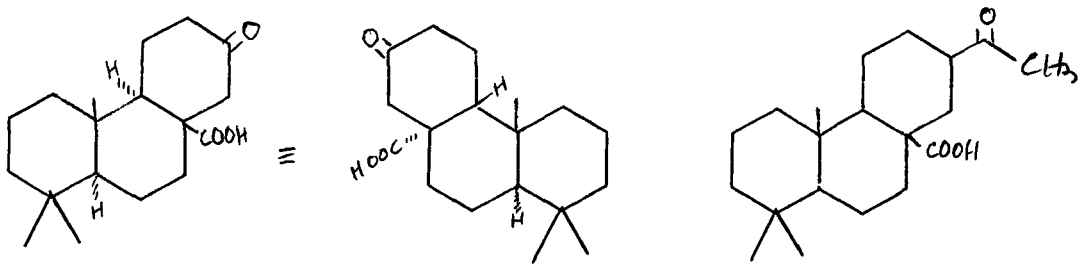
(XIV)



(XVa)



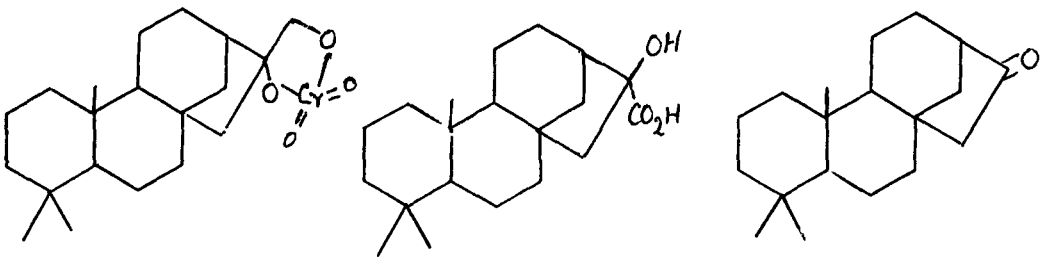
(XV)



(XVI) (XVIa) (XVII)

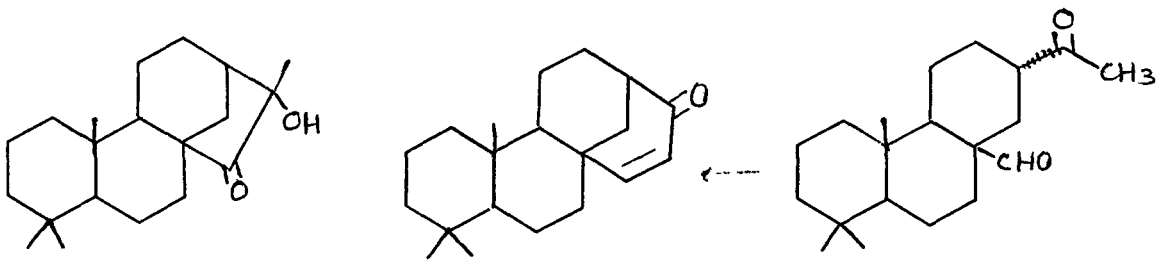
The diols obtained from phyllocladene and isophyllocladene should then be formulated as (XV) and (XVa), and the oxoacids as 13-oxo-8-β-carboxypodocarpane and 13-β-acyl-8-β-carboxypodocarpane (XVI) and (XVII) respectively.

Formation of the ketone $C_{19}H_{30}O$ can be rationalised on the basis of initial oxidation to the diol (XV) which undergoes further oxidation either by a cyclic transition state (XVIII) or through the hydroxy carboxylic acid (XIX) which loses CO and H_2O to give the ketone $C_{19}H_{30}O$ (XX).



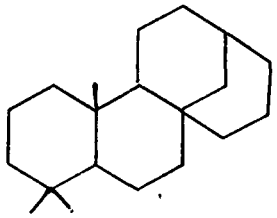
(XVIII) (XIX) (XX)

Further confirmation of the suggested structures was provided by the work of Grant and Hodges who prepared the above compounds under different and more specific conditions. Thus treatment of isophyllocladene with OsO_4 in ether/pyridine gave the diol (XVa) which was oxidised by CrO_3/H_2SO_4 to (XXI) and (XXII). The unsaturated ketone (XXII) is obviously formed through intramolecular aldol condensation of the ketoaldehyde (XXIII) the immediate product of the diol cleavage. It is accompanied by small amounts of the ketol (XXI).



(XXI) (XXII) (XXIII)

Wolff-Kishner reduction of (XXII) gave an unsaturated compound $C_{20}H_{32}$ which was hydrogenated over PtO_2 to D-homodihydro phyllocladane (XXIV). Lead tetraacetate oxidation of the diol (XVa) gave the keto aldehyde (XXIII) which was partially converted to (XXII) on chromatography over Al_2O_3. Complete conversion could be effected by treatment with 5% methanolic NaOH. Oxidation of (XXIII) with CrO_3/H_2SO_4 followed by treatment with methanolic HCl led to a mixture of the keto acids 13-α-acyl-8-β-carboxy podocarpane (XXV) and 13-one-8-β-carboxypodocarpane (XVI).



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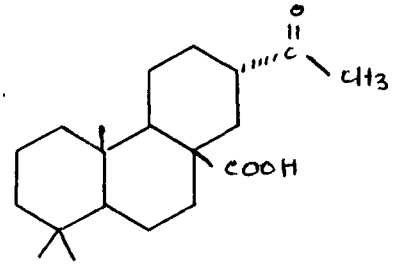
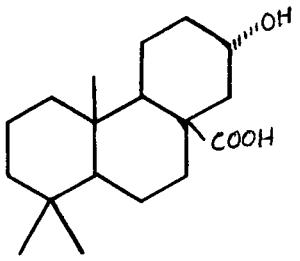


(XXV)

Baeyer Villiger oxidation of (XXV) with trifluoroacetic acid and H_2O_2 gave 13-α-hydroxy-8-β-carboxy podocarpane (XXVI) which was methylated with CH_2N_2 and oxidised with CrO_3/acetone to the methyl ester of (XVI). Of particular interest here was the correlation of (XXIII) to manool which was affected by the following sequence. (XXIII) was converted to the dioxime (XXVII) by $NH_2OH.HCl$/pyridine and the oxime dehydrated to the nitrile (XXVIII) with Ac_2O/NaOAc. Nitrous acid deoximation gave the parent ketone (XXIX) Baeyer Villiger oxidation of this with $CF_3CO_3H.HPO_4$ (anhydrous) gave (XXX) which was oxidised to the ketone (XXXI). HCN elimination from this by distillation under vacuum with powdered KOH gave the α,β-unsaturated ketone (XXXII) which was also prepared from manool (XXXIII) by a process not further specified in the abstracts (issues of Tetrahydron prior to 1961 were not available) but which should involve oxidation to (XXXII a) followed by intramolecular aldol condensation.



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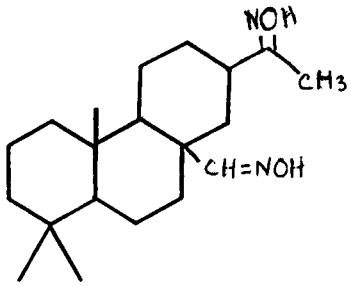

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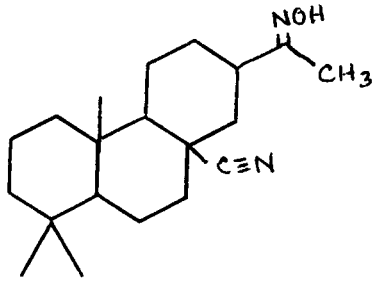

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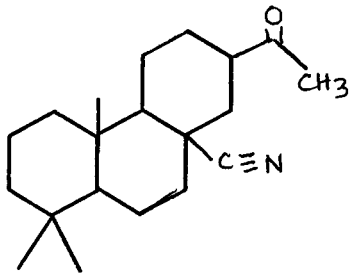

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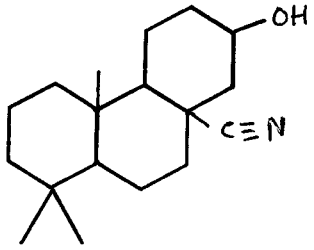

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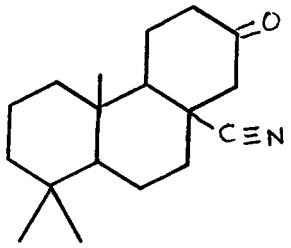

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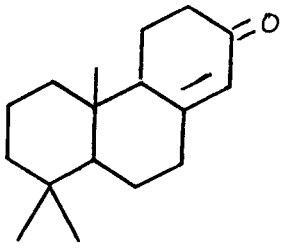

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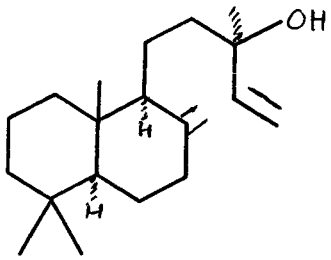

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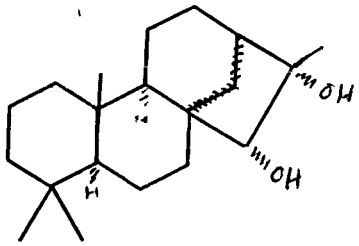
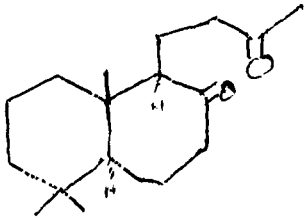
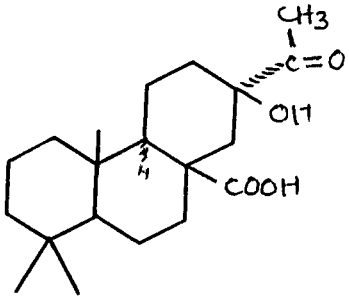


(XXXIV)



XXXIIa



(XXXV)

The absolute stereochemistry assigned to phyllocladene was based initially, in part, on its correlation to rimuene which was then considered to have one of the pimaradiene structure (V-VIII). Since rimuene was subsequently shown to have the structure (III) and further since GLC pure rimuene did not afford isophyllocladene this piece of evidence loses its validity.

However, since all naturally occurring diterpenoids have A B trans ring junction and since the unsaturated ketone (XXII) was related to manool (for which the absolute stereochemistry indicated in (XXXIII) was established by the method of extensive correlation studies based on molecular rotation differences) the trans fusion of these rings can be considered certain. The fusion of the five membered ring follows from ORD studies undertaken by Djerassi et al (13,14,15) in connection with this work on cafestol. These studies utilise the keto acid (XVI) which can also be written as (XVIa) and as such its ORD curve should correspond to 3-α-steroid 3-ketones e.g. cholestanone. It had been noted earlier that CO_2H behaves analogous to the methyl group in ORD correlations. The ORD curve of (XVIa) shows a positive cotton effect like that of 5α-3-keto steroids which favours a β-carbonyl and hence a β-fused 5-membered ring.

The absolute stereochemistry of the A B ring system as well as the β-fusion of the 5-membered ring was further confirmed by Ireland's synthesis of the derived ketone (XVI).

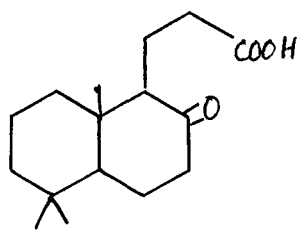
Much of Brandt and Hodges work was repeated by Briggs, Caine, Cambie and Davis (12) who also prepared some

other derivatives of interest. Thus the ketol (XXI) was obtained in better yields by them by Oppenauer oxidation of isophyllocladene diol (XVa), obtained by them through acetone permanganate oxidation of isophyllocladene. Since hydroxylation with this reagent and OsO_4 is a cis process and attack of the reagent is more likely to occur from the less hindered α face the glycol is formulated by them as (XXXIV).

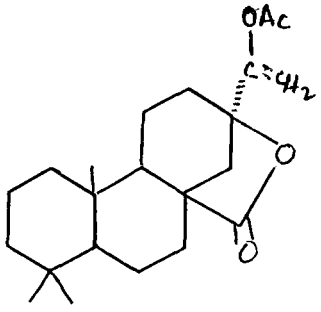
For similar reasons the ketoaldehyde (XXIII) is assigned the 13-α-stereochemistry owing to its stability towards acids and bases. Since fusion of the 5-membered ring has been shown to be β, epimerisation at this centre must have occurred during its formation.

Oxidation of isophyllocladene in dry acetone gave the acids (XXXV) and (XVI). The former was identical with material assigned structure (XXXVI) by Brandt. Structure (XXXV) follows from spectral data, besides iodoform reaction and sodium bismuthate oxidation to (XVI). The stereochemistry shown follows from its conversion with $Ac_2O/HClO_4$ to the enol lactone acetate (XXXVII).

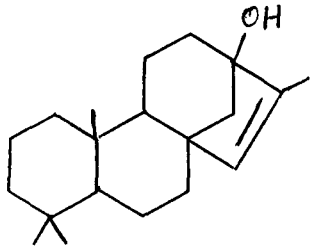
Formation of (XXXV) suggests initial allylic hydroxylation of isophyllocladene to (XXXVIII) followed by ring fusion.



(XXXVI)



(XXXVII)

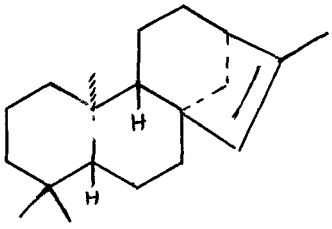


(XXXVIII)

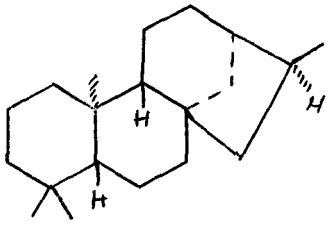
The stereochemistry of phyllocladene based on the correlation with manool and the above data was confirmed through its synthesis by Ireland. The absolute stereochemistry of manool was established as shown by Klyne (16) that of phyllocladene therefore also follows from the above correlation.

The above conclusions were further confirmed by the total synthesis of (±)-phyllocladene (17).

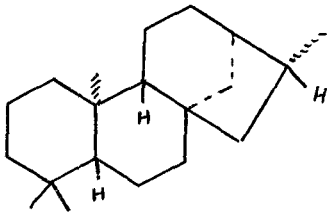
The other tetracyclic hydrocarbon kaurene was isolated initially from the leaf oil of the New Zealand kauri, Agathis australis and was shown to be stereoisomeric with phyllocladene (18). The kaurene (X), isokaurene (Xa) pair is distinguished by IR bands at 876 and 820 cm^{-1} attributable to the exocyclic methylene of kaurene and the trisubstituted double bond of isokaurene. Hydrogenation yields here again the C-16 epimeric pair of kaurane (Xb) and epikaurane (Xc).



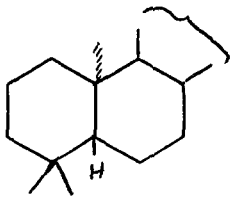
(Xa)



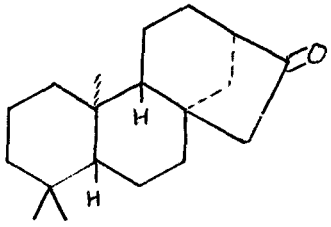
(Xb)



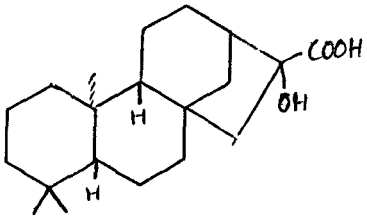
(Xc)



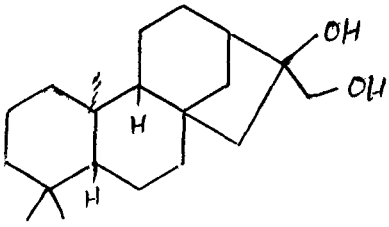
(Xd)



(XXXIX)

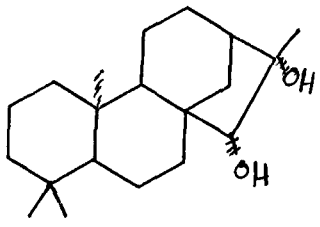


(XL)

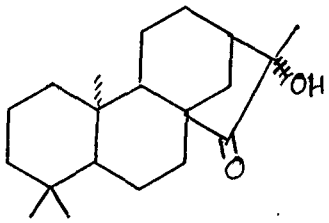


(XLI)

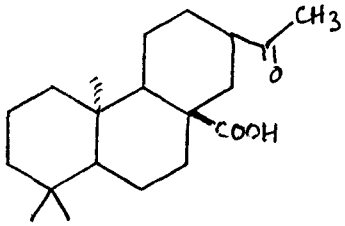
Acetone permanganate oxidation of kaurene afforded the nor ketone (XXXIX) and hydroxy acid (XL). OsO_4 oxidation of kaurene and isokaurene gave the corresponding diols (XLI) and (XLII) both of which are assigned the α-configuration on the basis of the absolute stereochemistry shown in (X) as hydroxylation should occur from the less hindered α face. Permanaganate oxidation of isokaurene gave products analogous with those obtained from isophyllocladene. The ketol (XLIII) showed hydrogen bonded hydroxyl at 3279 cm^{-1} and -C=O at 1736 cm^{-1} as required by this structure. The ketocarboxylic acid (XLIV) must have the acetyl and the CO_2H group on the same side, eventually shown to be β, since the IR spectrum in KBr (3279, 1719, 1692 cm^{-1}) showed that it existed in the solid phase as (XLV). In CCl_4 the keto acid tautomer (XLIV) predominates (1718, 1692, no OH absorption).



(XLII)



(XLIII)

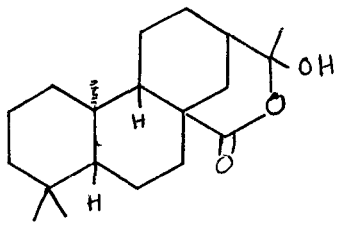


(XLIV)

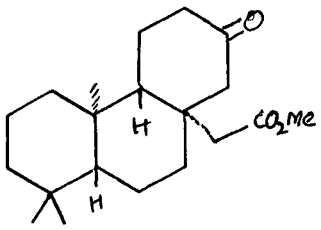
These transformations and degradations therefore establish that kaurene has the same carbocyclic ring system as, phyllocladene, as such, the difference between them must be of stereochemical nature.

Phyllocladene occurs with kaurene in Podocarpus ferruginens (11). Both are dextrorotatory and since phyllocladene has the normal steroid 5-α-10-β-stereochemistry (+) kaurene must also be similarly formulated. On this basis and the assumption of a trans anti backbone (-) kaurene can be represented by parts structure (Xd).

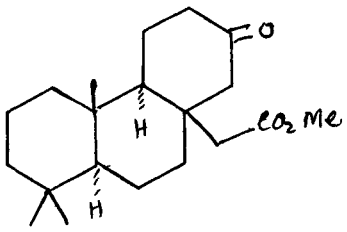
The fusion of the 5-membered ring is suggested by the positive cotton effects of the nor-ketones derived from phyllocladene and kaurene. This is further confirmed by the conversion of (-) kaurene and (+) phyllocladene into enantiomeric keto ester (XLVI) and (XLVII).



(XLV)



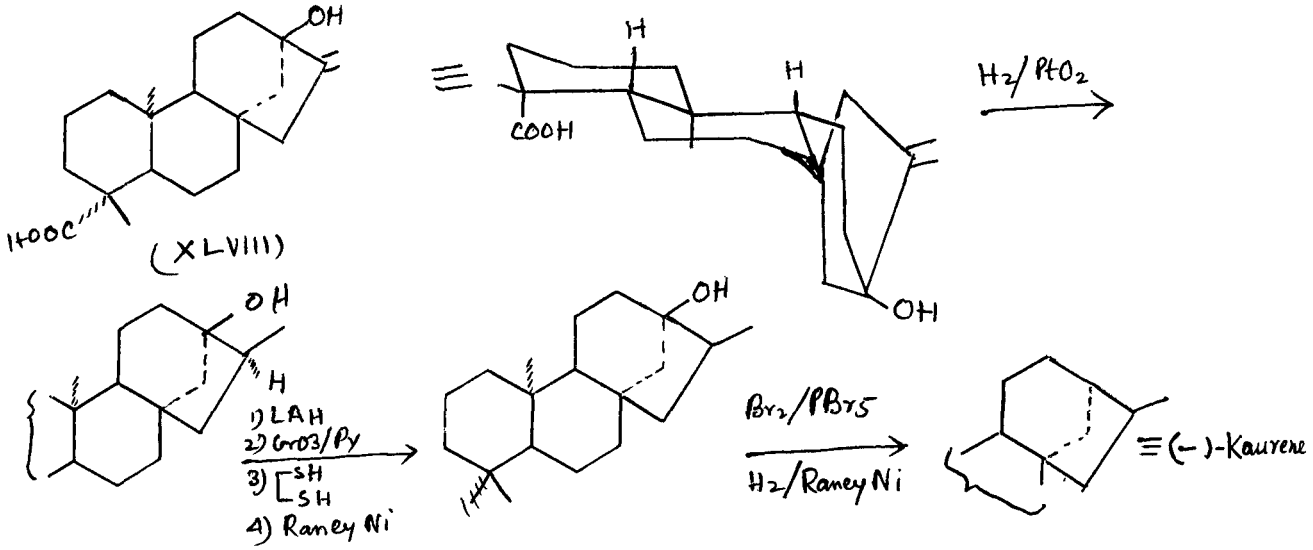
(XLVI)



XLVII

Another correlation of stereochemical significance is the conversion of steviol to stevane which was found

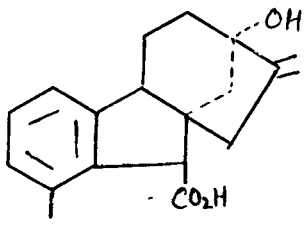
identical with (-) α-dihydrokaurene, the principal hydrogenation product of (-) kaurene. Steviol has been shown to have the structure and stereochemistry (XLVIII), the transformations leading to kaurene (19,20) are depicted in the sequence given below:



The stereochemistry of steviol is based on the comparison of the cotton effect curve of the derived nor ketone with that of the nor ketone derived from allogibberic acid (XLIX).

Further confirmation of these features comes from the synthesis of (±) kaurene (21).

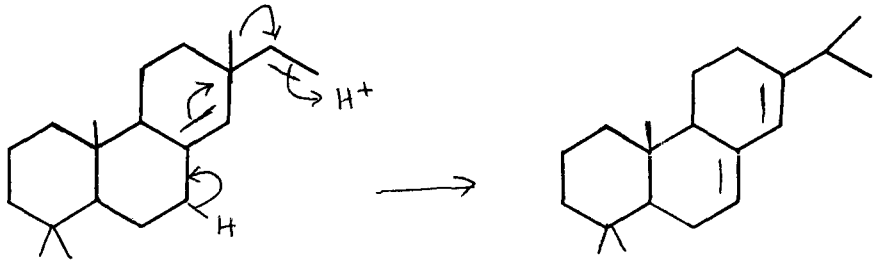
The stereochemistry of most tetracyclic diterpenoids including the Cerrye and atisine alkaloids, have been correlated and structures assigned can thus be accepted with certainty (22).



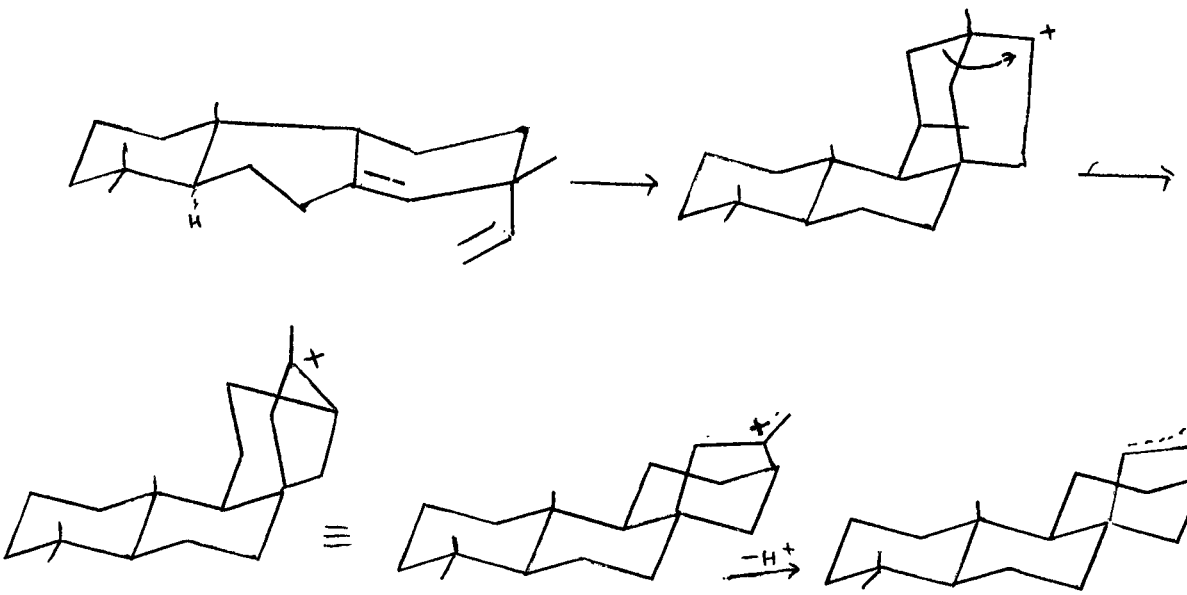
(XLIX)

Biogenetic consideration

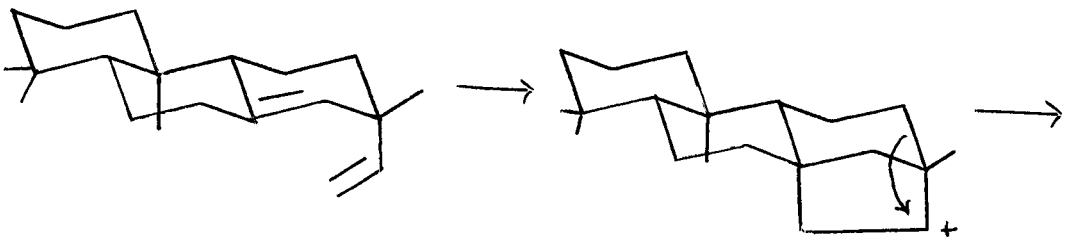
The evolution of tetracyclic diterpenes from tricyclic precursors was suggested by Wenkert who also took into account the steric and stereoelectronic requirement of the process. It had been pointed out by Ruzicka (23) that the pimarane should be the logical precursors of the abietanes, the terminal isopropyl group of the latter being formed by methyl migration as shown below for the formation of abietadiene from pimaradiene.

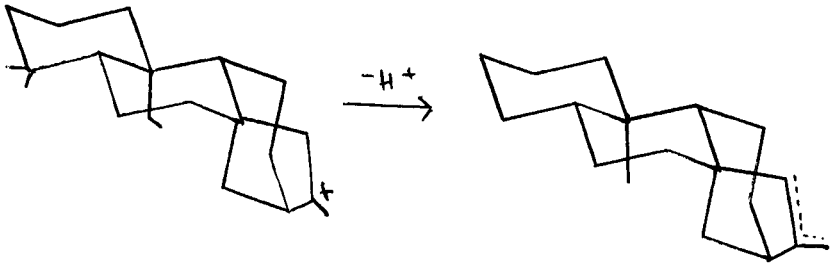


Wenkert postulated that this process should be facile only when the methyl group is in the sterically unfavourable quasi-axial orientation in which confirmation the stereoelectronic requirements would also be fulfilled. He went even further to suggest that all naturally occurring pimaradiene should have quasi-equatorial methyls. These compounds with quasi-axial vinyl group can undergo phytochemical protonation followed by bond migration to yield compounds with phyllocladene or kaurene skeleton depending on the nature of the pimaradiene involved in the transformation. Thus a phyllocladene would result from (L) and kaurene from (LL),



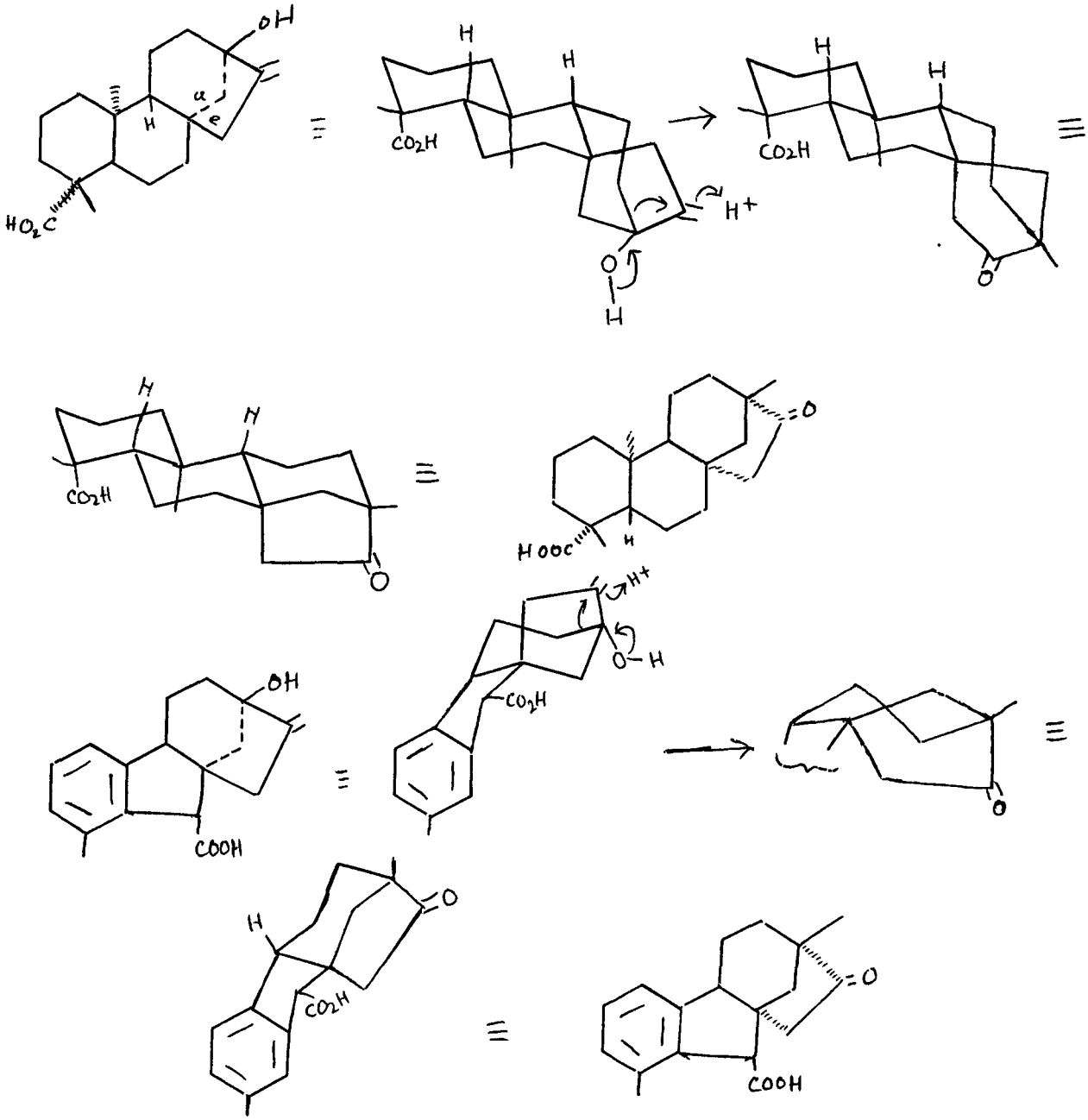
Phyllocladene or Isophyllocladene



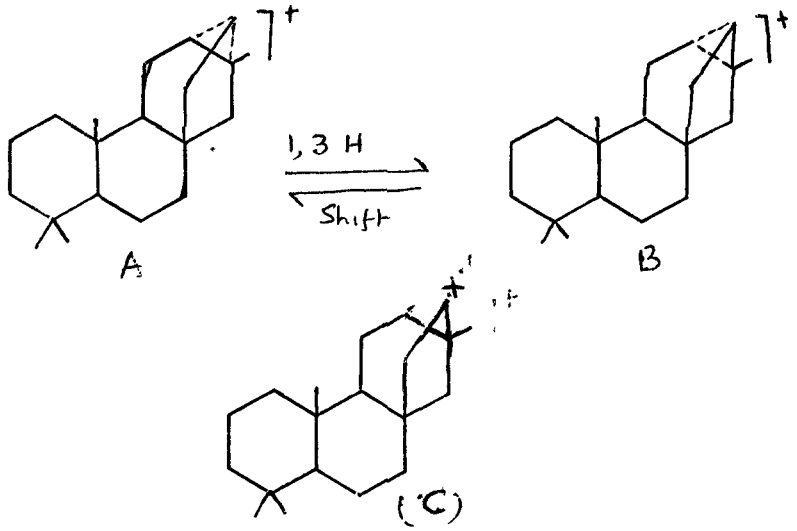


Kaurene or Isokaurene

these bond migrations and their stereochemical consequences are similar to the acid catalysed transformations encountered in the steviol, isosteviol and allogibberic acid, gibberic acid rearrangement (24).



The intermediate carbonium ion (Ω) should exist most probably in the nonclassical structure (A) and (B) or by analogy with similar ions in monoterpene chemistry as the tricyclonium ion(Ω).



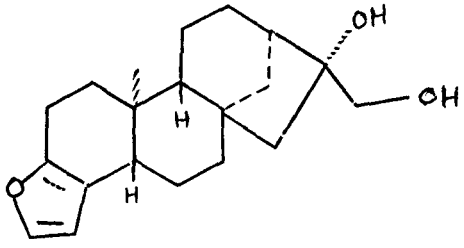
It needs to be noted here that Wenkert's prediction that all naturally occurring pimaranes would have equatorial C-13 methyl has not been borne out as pimaric acid has an equatorial methyl whereas sandaracopimaric acid has an axial methyl. On the other hand the suggested intermediacy of pimaradienes in the formation of tetracyclic compounds has been established through experiments with labelled compounds. Thus it was found that labelled (41) was transformed to 7-hydroxykaurenolide by Gibberella fujikuroi (25).

There is thus little doubt that a carbonium ion such as (C) is the precursor of all the tetracyclic diterpenes e.g. phyllocladane, kauranes, stachane and abietanes. Added support for this was provided by the isolation by Ourisson et al of the pentacyclic compound trichylobane (26).

Methods of structural elucidation

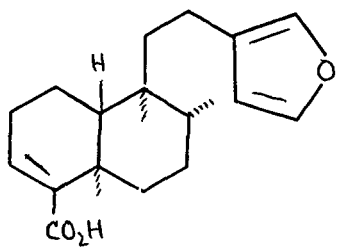
1. Nature of the carbon skeleton:

Classical methods of structure determination in this field have been reviewed by several authors (27,28,29). In earlier studies selenium dehydrogenation provided the most valuable evidence as regards the nature of the carbon skeleton. Oxidative cleavage to simpler fragments the nature of which could be inferred from analytical values and synthesis offered further insight into the carbocyclic framework and the location of substituents in it. Thus presence of part structure (LXI) in cafestol was deduced from lead tetraacetate oxidation to a ketone which was oxidised by potassium hypoidite to a dicarboxylic acid which formed an anhydride, a behaviour characteristic of dicarboxylic acids derived from 5-membered rings. Introduction of IR spectroscopy simplified such problems; the carbonyl group in 5-membered rings being characterised readily by the higher frequency 1740 cm^{-1} of the C=O stretching vibration in 5-membered ring ketones as compared to the C=O frequencies in 6 and higher membered rings. The UV and IR spectral measurements played a predominent role in structural

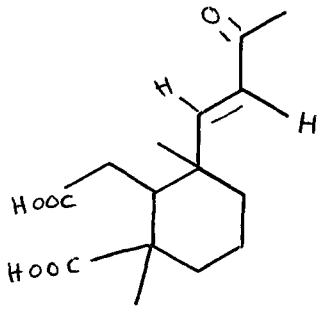


(LII)

elucidation till the advent of NMR spectroscopy. The NMR spectra of cyclic compounds e.g. terpenes and steroids are complicated and do not offer much information of value in determining the carbon skeleton if it is a new one. This limitation is responsible for the continued reliance on selenium dehydrogenation which can now however be conducted with milligram quantities because of the efficiency of GLC in resolving and identifying the mixture of aromatic hydrocarbons produced in the reaction. Even so NMR spectra are frequently a valuable guide in fixing up the position of the methyl groups and, of course, in establishing their number. This can be illustrated by consideration of the NMR spectrum of rimuene (LII) in which a methyl group at 9 rather than the usual 10-position is suggested by the high field value (9.38τ)* of one of the methyls as well as by the presence of allylic coupling in the signal of the olefinic protons. Another suitable example is that of hardwickiic acid (LIII) (30) in which a methyl group was placed at C-9 because of the doublet of the adjacent olefinic proton in the degradation product (LIV).



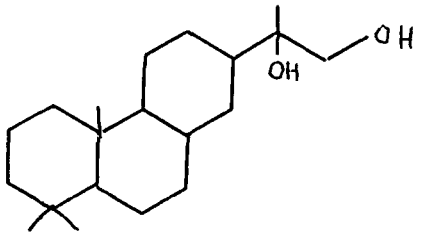
(LIII)



(LIV)

*τ values are used throughout the thesis.

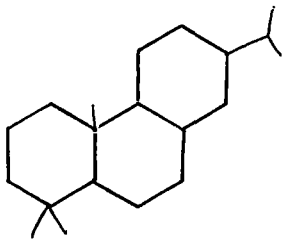
Above all the NMR spectra are helpful in establishing the position of substituents once the carbocyclic framework is known. With the number of diterpenes now known this is very frequently the case. The comparative data available from literature serves also to distinguish the carbon skeletons of known compounds though it is advisable to check the validity of such deductions by correlating the product under study to a known derivative based on the assumed carbon skeleton. Thus a kaurane e.g. (XLI) can be readily distinguished from a similar abietane derivative (LV) by simply counting the methyl signals in the spectrum. However, there is no way



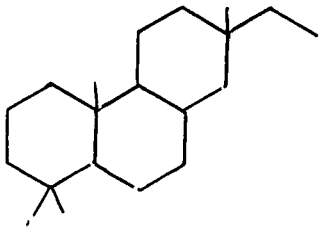
(LV)

of distinguishing between the isomeric kaurane and phyllocladene diols (XLI) and (XV).

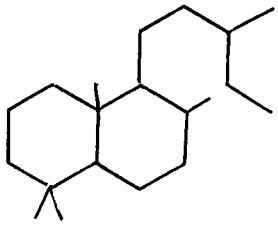
Similarly derivatives of abietane (LVI) pimarane (LVII) and labdane (LVIII) can be readily distinguished among themselves by number and multiplicities of the methyl resonances, the functional groups e.g. OH, CeO, COOH assisting rather than hindering in such assignments by causing predictable shifts in the position of the methyl signals on account of the magnetic anisotropy introduced by their presence.



(LVI)

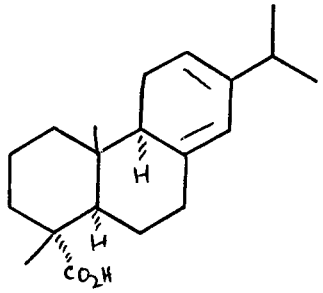


(LVII)

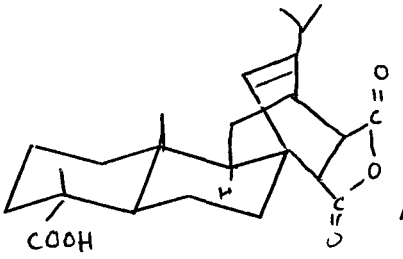


(LVIII)

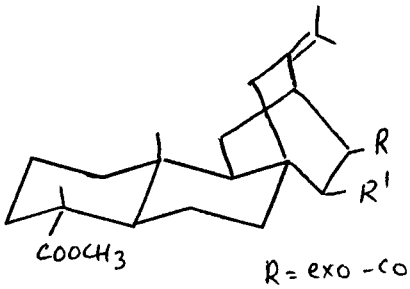
The anisotropy of the C=C double bond is similar to that of the carbonyl group which also helps in locating the methyl groups. Thus levopimaric acid (LIX) was erroneously assigned the 9-β-configuration by Klyne (16) employing the method of molecular rotation differences. NMR studies on the maleic acid adduct (LX) and its double bond isomer (LXI) revealed (31) the configuration at this centre to be α as suggested by Burgstahler *et al* and Pople (32,33). This follows from the observation that the C-10 methyl resonance in (LX) occurs upfield at 9.41 as compared to 9.19 in (LXI) where the exocyclic double bond does not fulfill the geometrical requirements for interacting with the C-10 methyl.



(LIX)

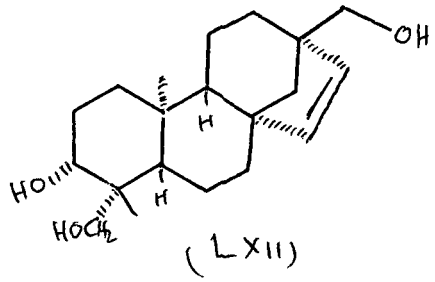


(LX)



(LXI)

R = exo -$COOCH_3$
R' = endo -$COOCH_3$



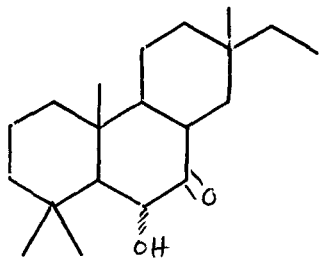
(LXII)

This long range shielding is exercised by the double bond on protons which lie directly above and below the trigonal plane of the carbons. The nuclei which lie in or near the trigonal plane are deshielded. In more specific terms an olefinic centre attached to C-8 shields the C-10 methyl if the geometry of the molecule is such that it lies above or below the trigonal plane. This requirement is fulfilled by the trans anti trans backbones of beyrol (LXII) and isophyllocladane (IV) in both of which the C-10 methyl is shielded while no change in the value of this methyl is observed in isokaurene (Xa) which has a trans anti cis backbone. Significant shielding requires that the methyl group should not be more than 3 A° removed from the centre of unsaturation (34).

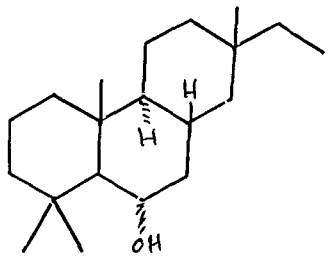
It is thus apparent that indirect NMR spectral evidence can lead to the assignment of the correct carbon skeleton to an unknown derivative based on a known carbon skeleton. However, because of the additivity of the opposite effects i.e. shielding by some substituents and deshielding by others one has to be cautious in making such deductions from the NMR spectrum.

2. Location of the functional groups[1]

There are two ways in which the position of a functional group e.g. OH or COOH in a diterpene is indicated by the NMR spectrum. Firstly, the position and multiplicities of the adjacent protons and secondly the shielding or deshielding of other groups in the molecule by the substituent. Thus a carbonyl at C-7 in pimarane derivative (LXIII) can be inferred from the position of the doublet and the multiplets of the

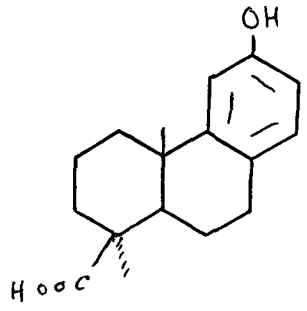


LXIII

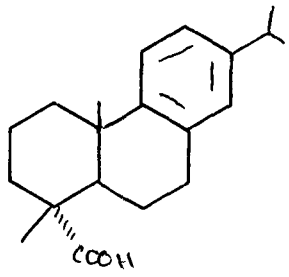


LXIV

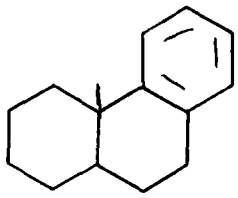
adjacent 6 and 8 protons as well as by the deshielding compared to (LXIV) of the C-10 methyl. The earliest studies of this nature were conducted by Wenkert (35,36) and his associates on derivatives of podocarpic acid (LXV) dihydro abietic acid (LXVI) and a synthetic analogue (LXVII). In a subsequent paper the spectrum of pimarane derivatives were also studied.



LXV



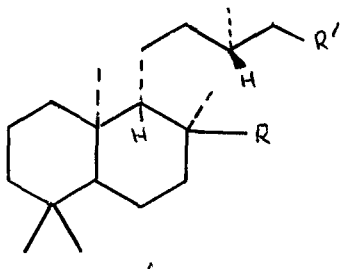
LXVI



LXVII

The effect of subtle conformational features on the resonances of methyl groups is shown by comparison of the position of the C-10 methyl resonances in (LXVI) and (LXVII). The resonances of this methyl in (LXVII) occurs at 8.93 and (LXVI) at 8.78. The deshielding of this methyl in (LXVI) is due to the distortion in bond angles brought about by the substituents on C-4 which forces ring A to assume a half boat conformation which places the methyl group in the deshielding zone of the aromatic ring. Similar comparison between (LXVI) and (LXV) shows the shielding effect of the axial C-4 carbonyl on the C-10 methyl. This shielding is experienced by any given methyl in 1,3 diaxial relationship to a CHO, COOH, COOR or CN group. The C-4 methyl itself is however deshielded by the neighbouring CH=O, COOH, COOR and CN function by 0.23, 0.34, 0.39 and 0.46 ppm.

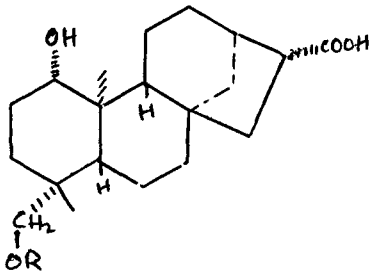
The spectra of labdane derivatives were investigated by P.R.Jefferies (37). As an example the NMR spectrum of the diol (LXVIII) shows the presence of 4-methyl groups at 9.19 (6H), 9.13, 8.85 (s, 3H each) and 9.09 (d, J=6 c/s). The $-CH_2CH_2OH$ grouping is characterised by a symmetrical two proton triplet at 6.33 (J=6 c/s).



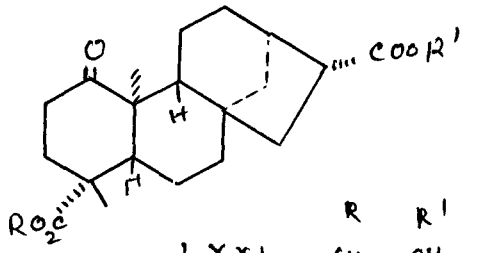
R'=R=OH

LXVIII

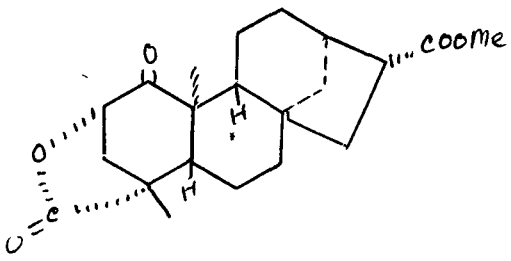
The NMR spectra of a number of kauranoid diterpenes have also been reported by these authors (30). The resonances of the methyl groups in compounds studied by them are given in the table below:



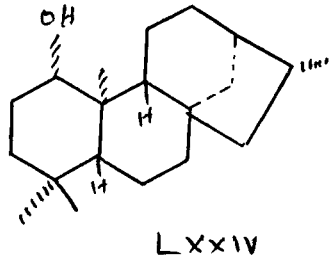
LXIX R = H
LXX R = Ac



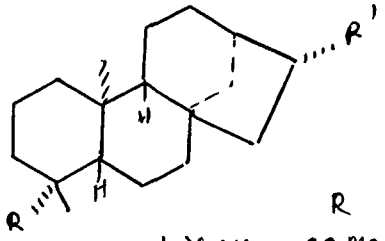
	R	R'
LXXI	CH_3	CH_3
LXXII	H	CH_3



LXXIII



LXXIV



	R	R'
LXXV	CO_2Me	CO_2Me
LXXVI	CH_2OH	CO_2Me
LXXVII	CH_3	CH_3
LXXVIII	CH_2OAc	CH_2OAc
LXXIX	CO_2H	CO_2H

Chemical Shifts (values) of Methyl Groups in Kauranes Derivatives

Compound	Substituents 4-Axial	1	4-Methyl	10-Methyl
LXXVI	CH_2OH	H	9.04	9.01
Methyl ester of LXIX	CH_2OH	OH(∝)	9.07	8.91
LXXVII*	CH_3	H	9.13,9.19	8.99
LXXIV	CH_3	OH(∝)	9.19,9.20	8.88
LXXVIII	CH_2OAc	H	9.06	8.97
LXX	CH_2OAc	OAc(∝)	9.06	8.79
LXXIX	CO_2H	H	8.77	9.07
LXXII	CO_2H	=O	8.68	8.64
LXXV	CO_2Me	H	8.83	9.18
LXXI	CO_2Me	=O	8.78	8.78
LXXIII	CO_2Me	=O	8.76	8.49

* 16- -Methyl, 9.09 (d, J=7 cps).

On the basis of the data given above and available generally from literature the following points deserve to be emphasised.

The hydroxymethyl group on C-4 can be either axial or equatorial. Provided that C-3 is not substituted

the two types can be distinguished, the quartet of the axial hydroxymethyl group being centred at 6.2 and that of the equatorial at 6.65 (39). Both quartets are shifted paramagnetically to 5.8 and 6.25 respectively on acetylation (40,41).

Further support for these assignments can be obtained by oxidation to the aldehyde. When the aldehyde function is equatorial the aldehydic proton appears at 0.77 whereas when it is axial it appears at 0.23 (42).

Axial and equatorial acids and esters

The deshielding of the angular C-10 methyl by axial carboxyl and carbomethoxy groups on C-4 has already been referred to. This effect is also evident from a study of the NMR spectra of several kaurane derivatives having different substituents on C-4 reported by P.R.Jefferies (40). It has been used in making configurational assignments on C-4 but to do this the chemical shift of the C-10 methyl in the parent compound must be known. When this is not the case reduction of CO_2R to CH_3 involving several steps is necessary.

In such cases a more convenient and direct procedure introduced by C.R.Narayanan (43) can be used. This is based on the fact that since presence of a carbonyl or a carbomethoxy function in 1,3-diaxial relationship to a methyl leads to shielding of the methyl group the anion of the acid must deshield in the same relationship. The correctness of this assumption was established by measuring the spectra of 10 acids and their esters in pyridine.

Methylene adjacent to carbonyl:

The interpretation of the signals of the methylene protons adjacent to a carbonyl function is difficult owing to the long range effects of the carbonyl group (44) and the complex spin interactions of these protons with protons on neighbouring carbons. The base induced exchange of these protons with deuterium is frequently helpful in establishing the position of the carbonyl group in the molecule e.g. macarangenol (45).

DISCUSSION

Callicarpa macrophylla (Verbenaceae):

Callicarpa macrophylla is a shrub growing in the upper gangetic plain, from Kashmir in western Himalayas to Assam in the east, reaching up to a height of 6,000 ft. The plant material used in the present investigation was collected from Dehra Dun in central north India and was identified by the Botanical Survey of India, Dehra Dun. According to Nadkarni (46) it is used in the treatment of rheumatic disorders only but from the information provided by the B.S.I. and the Forest Research Institute, it appears that it is held in high regard by the local people as a remedy for oral infections and intestinal disorders.

Though there was no report of any previous work on the extractives of this plant when the present study was undertaken other species of the genus Callicarpa, e.g. Callicarpa candicans (47), Callicarpa japonica (48) and Callicarpa maingayi (49) had been investigated recently and the existence of flavonoid and diterpenoid constituents in them was established.

Callicarpa macrophylla grows mostly in swampy areas which made it difficult to collect more than ~ 2 kgs of the dried plant in two visits to Dehra Dun.

The solid obtained from the light petroleum extract of the plant was shown by TLC to be a mixture of two components. Separation of these was accomplished by column chromatography over silica gel which afforded two crystalline compounds m.p. 123° and 133° designated at the time as CM-1 and CM-2 respectively because of the order of their elution from the column.

Elementary compositions

Elemental analyses of CM-1 and 2 agree with the molecular formulae $C_{22}H_{34}O_4$ and $C_{20}H_{32}O_3$ respectively. The difference of C_2H_2O between the two formulae suggested that CM-1 is the acetate of CM-2. This conclusion was supported by the spectral data presented below:

Spectral data:

Ultraviolet: Neither of the two compounds has a significant UV absorption above 210 nm but the spectrum of CM-2 shows a slight absorption at 280 nm agreeing in intensity with the absorption resulting from the n- π^*-transition of the isolated >C=O chromophore.

Infra-red: The IR spectrum (Fig.1) of CM-2 shows a very broad hydroxylic band between 3300-3400 and a sharp carbonyl band at 1708 cm^{-1}.

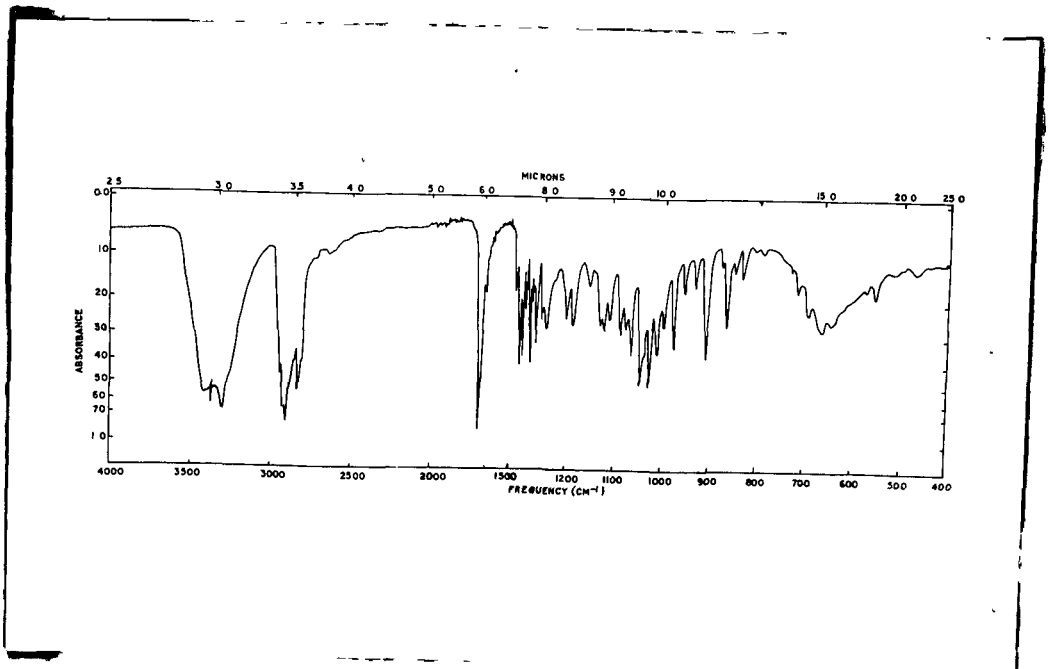


FIG 1

NMR: The NMR spectrum of CM-2 (Fig.2) shows resonances from three tertiary methyls at 8.92, 8.97 and 9.02 and the broad hump of the methylene protons, a characteristic feature of the spectra of polycyclic systems, between 8.2 to 8.7. The downfield quartet with very pronounced inner lines can only be assigned to two mutually coupled protons of a methylene group owing to the large coupling constant (J_{gem} = 11 cps) of the concerned protons. The chemical shifts, 6.16 and 6.38, of the two AB doublets responsible for this quartet suggest that the methylene group is bonded to an oxygen atom and the absence of any further interaction requires its attachment to a tertiary carbon in the molecule. There are several unresolved signals between 7.4 and 8.0. The rise of the integral over this region corresponds to about 7 protons, taking the downfield multiplet as a reference.

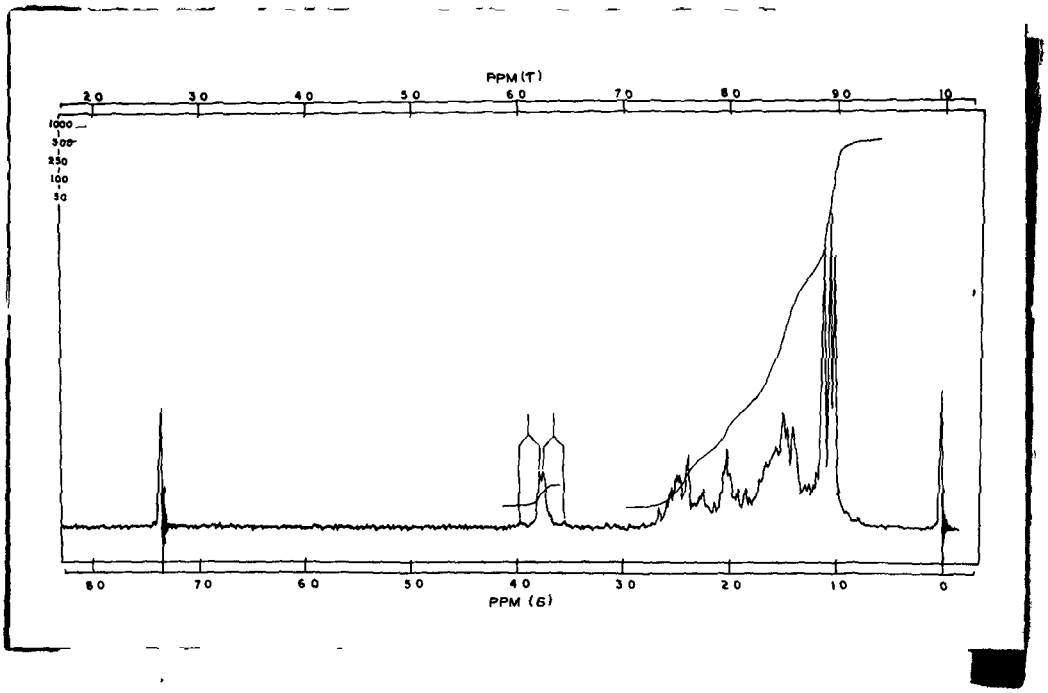
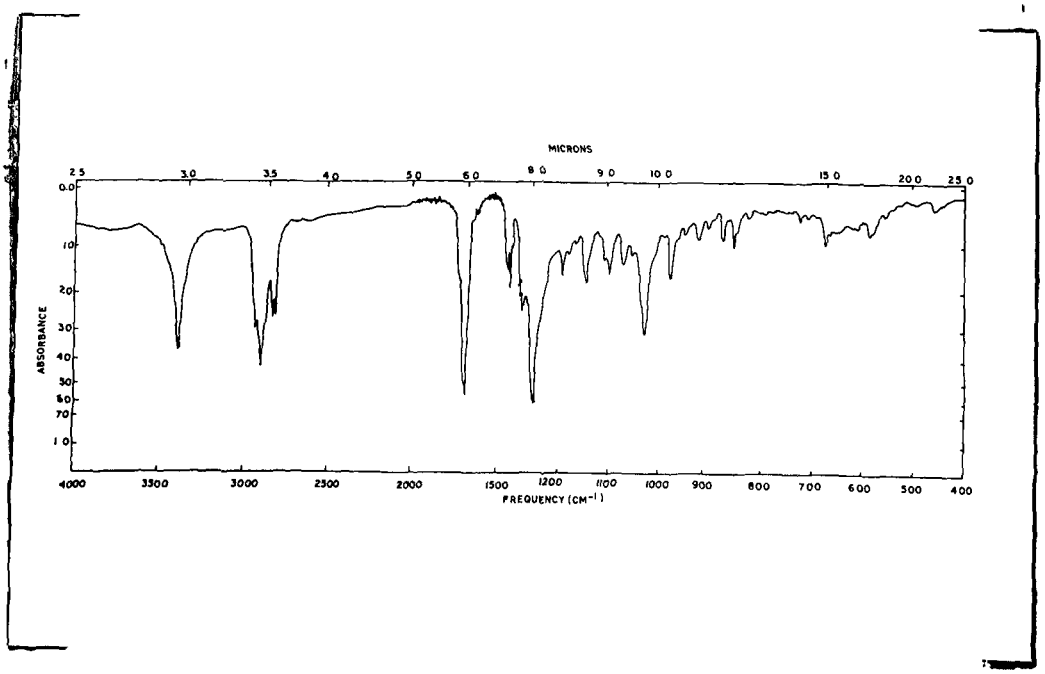


FIG.2

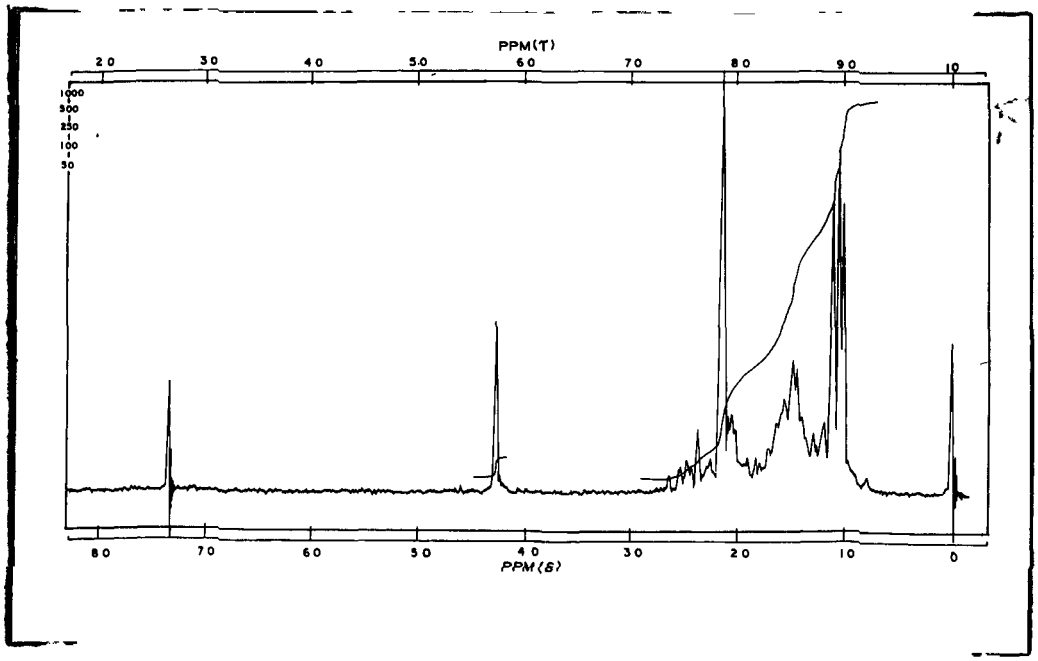
The spectral evidence discussed above establishes the presence of a ketonic function in a six or higher membered ring since a side chain carbonyl would have given rise either to a sharp singlet from the R-$\overset{O}{\overset{\|}{C}}$-$CH_2$-R group or a well resolved triplet from the R-$\overset{O}{\overset{\|}{C}}$-$CH_2$-$CH_2$-R group. The presence of atleast one methylene adjacent to the carbonyl group is demonstrated by the pink colour produced in the Zimmerman test (30). The presence of a primary alcohol function is also made very likely by the quartet of the R-CH_2OH protons and the broad hydroxyl band in the IR spectrum. The other features of the NMR spectrum can be more clearly discussed along with the spectral data of CM-1.

CM-1: IR: The IR spectrum (Fig.3) of CM-1 shows hydroxylic absorption at 3390 cm^{-1}. The carbonyl band at 1702 seems to have contributions from three carbonyl groups if breaks in the downward slope of the band at 1713 and 1725 cm^{-1} are taken into account. The intensity of the band, however, is not



sufficient for three carbonyl groups and a more likely interpretation is that the carbonyl band at 1708, (1713 + 1702/2 = 1708) is split and there are thus only two carbonyl groups in the compound having absorption at 1725 and 1708 cm^{-1}. The 1725 cm^{-1} band can be assigned to the acetate carbonyl since there is a pronounced -C-O-C- stretching band at 1245 cm^{-1}.

The NMR spectrum of CM-1 (Fig.4) resembles that of CM-2 except for the presence of an acetate methyl signal at 7.88 and the replacement of the quartet of the methylene protons by a sharp singlet at 5.79. The methyl resonances are approximately at the same values, 8.91, 8.96, 9.0 as in CM-2. The number of deshielded protons between 7.3 to 8.0 works out to about three but there is no recognisable pattern of coupling. The spectroscopic evidence therefore supports the conclusion drawn from the elemental analysis that CM-1 is the acetate of



CM-2. This was confirmed both by conversion of CM-1 to CM-2 by base hydrolysis and of CM-2 to CM-1 by acetylation.

The molecular formulae, spectral characteristics and the establishment of the above relationship between CM-1 and CM-2 shows both compounds to be diterpenes. Since acetylation of CM-2 results in the formation of only a monoacetate, identical with CM-1, which still has a hydroxyl band, CM-2 must be a dihydroxy compound. The hydroxyl groups must be tertiary and primary to account for the resistances to acetylation of one hydroxyl and the quartet observed in the NMR spectrum of CM-2. The presence of a ketonic function is definitely established by the IR spectrum and all the three oxygens are thus accounted for. This means that on the basis of its molecular formula $C_{20}H_{32}O_3$, CM-2 must be tetracyclic.

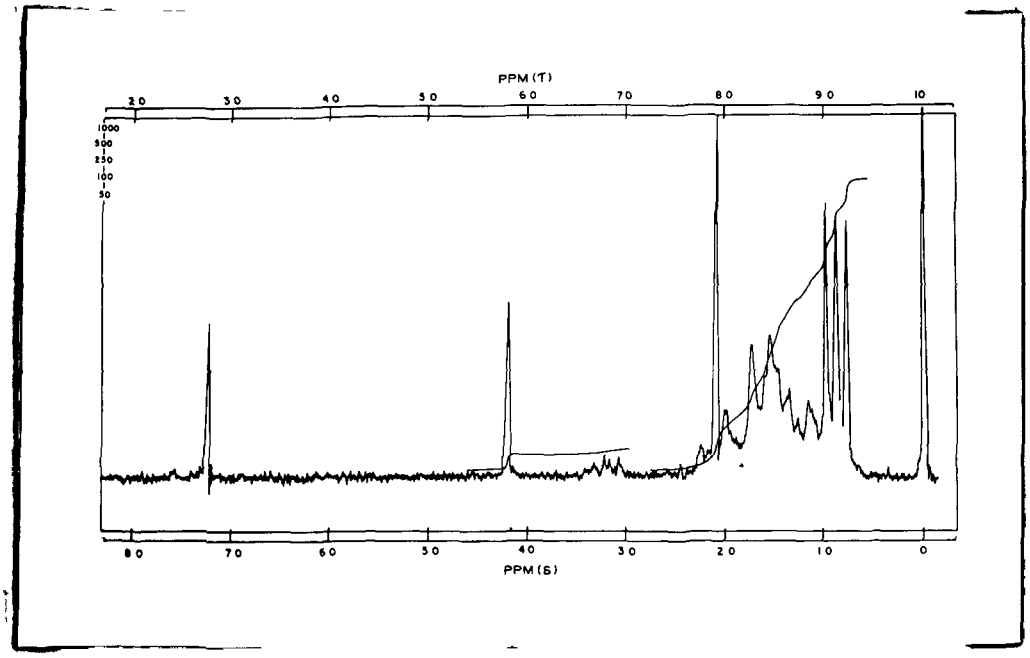
The most well known members of this group of diterpenes are derivatives of the stereoisomers kaurane and phyllocladane. The derivatives of stachane are far less numerous and have so far been isolated only from conifers and plants of the Euphorbiaceae family. Since existence of one of the tertiary methyls, is established there should be only three tertiary methyls left on the assumption of the kaurane or phyllocladane carbon skeleton. The presence of three tertiary methyl signals in the NMR spectrum fits in with this interpretation. It is not possible on the basis of available spectroscopic evidence to distinguish between these two stereoisomers and the choice in favour of one or the other can be made only through correlation with a known kaurane or phyllocladane derivative. The problem of structural

elucidation would then be reduced to the assignment of functional groups in the phyllocladane or kaurane nucleus. Subsequent experiments were therefore designed to obtain a derivative which can be compared with a known compound. This requires that some preliminary information be available about the location of the functional groups in the tetracyclic carbon framework so that appropriate experiments can be devised for conversion of CM-2 to a compound of established structure and stereochemistry. Some clues to the possible location of the primary hydroxyl and the keto function are provided by the NMR spectra of CM-2 and its lithium aluminium hydride reduction product.

The signal of the $\underline{CH_2}$-OR protons appears as an AB quartet in the alcohol and as a singlet in the acetate. This would seem to rule out a hydroxymethyl group at C-4 as the methylene protons of both the axial and equatorial CH_2OAc group attached to C-4 appear as a quartet though, of course, shifted downfield compared to the methylene protons of the alcohol. This behaviour has however been reported only for compounds which do not have a carbonyl group at C-3, and it is conceivable that the distortion of ring A produced by the C-3 carbonyl might influence the signal of the CH_2-OAc protons. This effect should be cancelled out on reduction of the carbonyl to hydroxyl since oblongifolicl (51) behaves in the normal way and its NMR spectrum displays a quartet of the geminal methylene protons.

Reduction of CM-2 with lithium aluminium hydride afforded a triol m.p. 260° the primary hydroxy group of which was acetylated selectively by brief treatment with acetic anhydride/pyridine at 0°C. The NMR spectrum of this (Fig.3)

shows the CH_2OAc protons again as a sharp singlet at 5.84.



Hence an adjacent carbonyl can not be the cause of the observed equivalence of the methylene protons and a CH_2·OH group at C-4 can be ruled out conclusively.

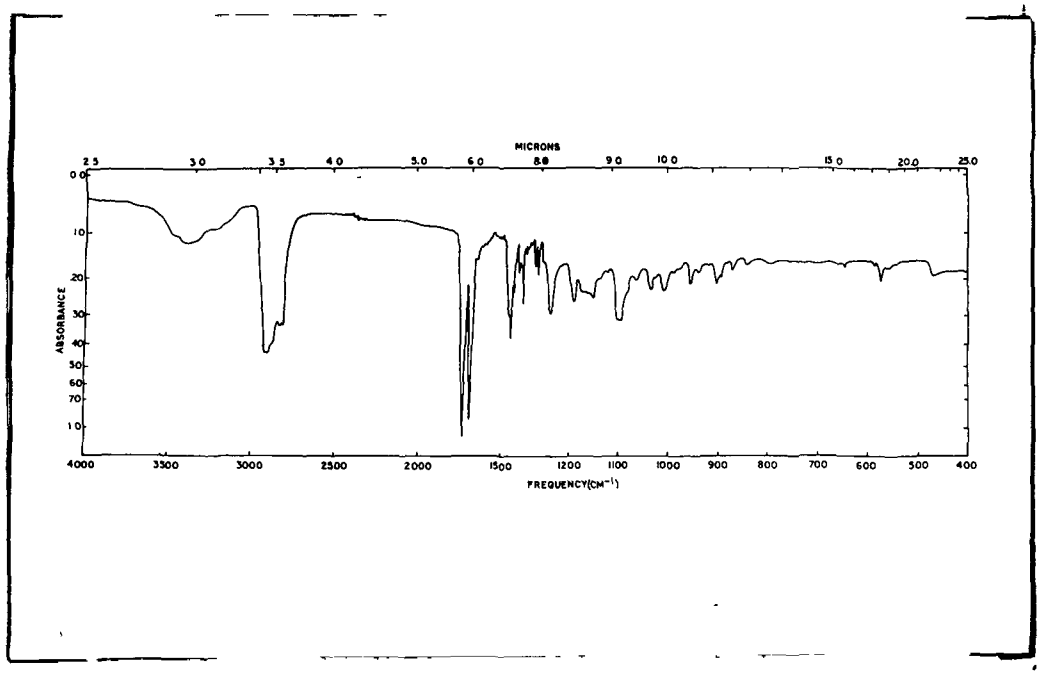
The NMR spectrum of the triol monoacetate is characterised by the upfield shift of the methyl resonances which now occur at 9.02, 9.2 and 9.3. The deshielding of the tertiary methyls by the carbonyl group in cyclic systems e.g. steroids, terpenes etc. is a well established fact and the upfield shift of the methyl resonances on reduction of the carbonyl group is therefore

understandable. Much information can be derived from a comparative study of the position of the methyl resonances in different derivatives of CH-2 and of other diterpenes with similar structural features but this is more usefully done after discussing the spectral data of the other compounds. Confining the discussion at this stage to the position of the hydroxymethyl group an alternate location for this group in the kaurane skeleton is C-16. The C-17 protons in eorymbol (52) diacetate which has a hydroxyl at C-16, and thus fulfills the requirement of attachment of the hydroxymethyl group to a tertiary centre, appear as a singlet at 5.77 . In eorymbol triacetate the CH_2-OAc protons give rise to the usual AB pattern but this has been ascribed to the hinderance to free rotation produced by the interaction between the neighbouring acetate functions.

Another compound of the same type is abbeokutone (53). Here again the C-17 protons appear as a singlet both in the alcohol and the acetate. These comparisons make it very likely that the hydroxymethyl in CH-2 is attached to C-16 which carries also the tertiary hydroxyl.

Chemical support for this was readily obtained by periodate oxidation of CH-2 which afforded a diketone showing (Fig.6) carbonyl absorption at 1735 and 1700 cm^{-1} the former band establishing the formation of a 5-membered ketone in the reaction.

A number of naturally occurring tetracyclic diterpenes have the 16-17-dihydroxy kaurane structure, enfentol (27) being the first such compound to be isolated. It seemed therefore at this stage of the work that CH-2 also

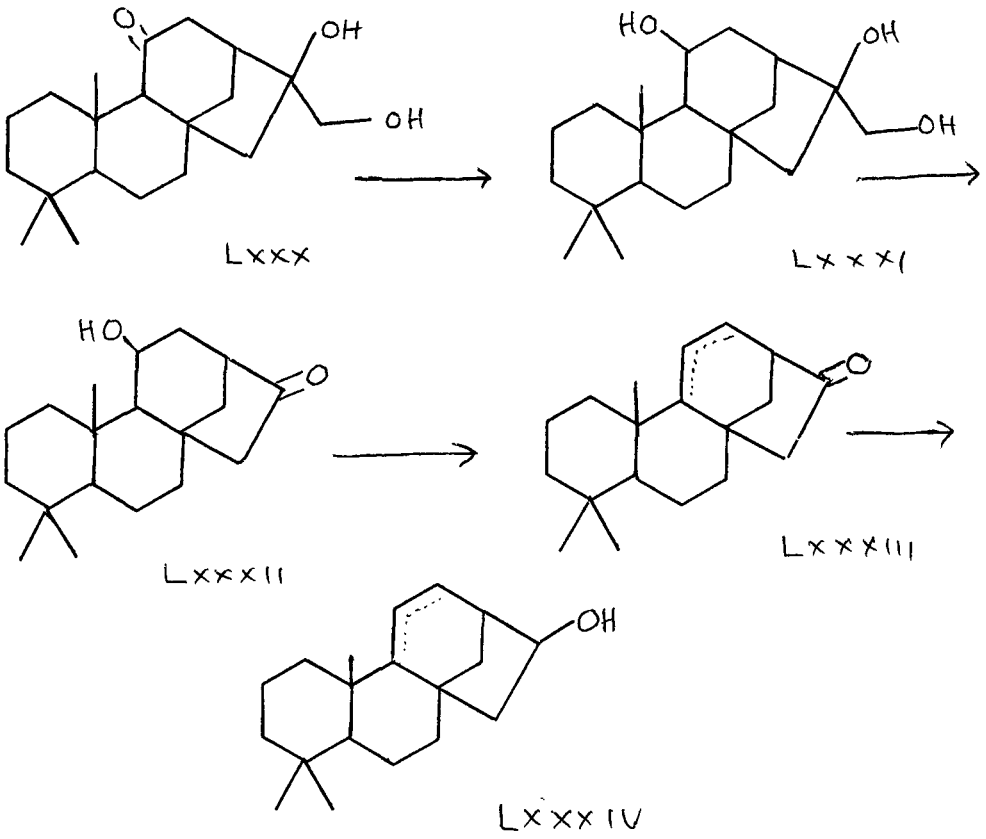


belong to this group and further confirmation of this was sought to be obtained by elimination of the carbonyl oxygen. This could be done either by Huang Minlon reduction or by reduction to alcohol followed by dehydration and if a mixture of olefins was formed, hydrogenation. The latter reaction could be applied only to (LXXXII) obtained by reduction and periodate oxidation of CM-2 since otherwise involvement of the primary and tertiary OH groups would have led to mixtures and the results would have been ambiguous.

The Huang Minlon reduction was attempted first employing conditions reportedly used in the case of abbeokutone but this resulted in formation of a mixture of three products. The predominant component of the mixture could be obtained pure by column chromatography but it turned out to be identical with the triol obtained earlier by lithium aluminium hydride reduction of CM-2. This suggested that the carbonyl group was hindered as reduction to alcohols has been observed under Huang Minlon conditions in the case of hindered ketones.

By analogy with steroids and triterpenes the most hindered positions in a phyllocladane or kaurane skeleton should be 11 and 6. The 3 position is comparatively less hindered but carbonyl groups at this position are known to react abnormally also as in the abnormal Beckman rearrangement. Since 3 and 6 exo-16-17-dihydroxy kauranes are known the 11-oxo-kaurane structure for CM-2 appeared most likely on the assumption of a kaurane skeleton.

Added support for the presence of oxygen at C-11 seemed to be provided by the isolation of calliearpone (47) from <u>calliearpa sandiensis</u>. Since a hydroxyl at C-11 can be reduced only under completely anhydrous conditions, which are not easily accomplished, the Huang Minlon reduction was not pursued further at this stage and the sequence shown in (LXXX to LXXXIV) was applied (the C=O is shown tentatively at C-11).Conversion of (LXXXIII)to(LXXXIV) was necessary as the dehydration product was an oil which could not be purified by repeated crystallisation and

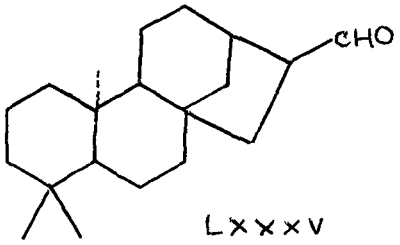


LXXX LXXXI LXXXII LXXXIII LXXXIV

GLC facilities were not available. The NMR spectrum of this product showed that it was a mixture, the olefinic proton integrating for less than one proton and there being apparently more than 3-methyls in the compound. Hydrogenation of the olefinic mixture may have resulted in formation of a single product but the compound was unaffected after 3 hrs treatment with hydrogen over Pd/c.

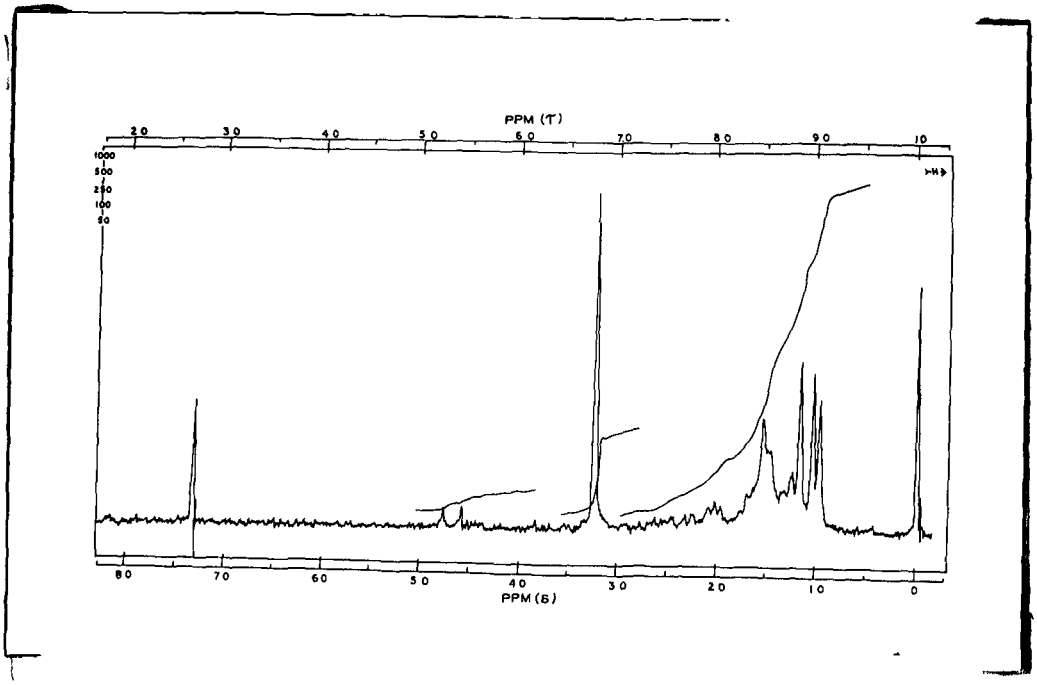
Another reaction frequently used for effecting the change $>C=O$ to $>CH_2$ is Raney nickel desulfurization of the

ethylene thioketal. When applied to abbeokutone it afforded first the bisethylene thioketal of the aldehyde (LXXXV) which can be formed through initial BF_3 catalysed elimination of the tertiary hydroxyl. Desulfurisation of the thioketal (LXXXV) in refluxing dioxan over Raney nickel then gave (-) kaurane.



LXXXV

Treatment of CH-2 with ethane dithiol under conditions identical with those employed in the case of abbeokutone gave a product m.p. 180° which was subjected to Raney nickel desulfurisation. An amorphous solid, m.p.90-100° resulted from this reaction the IR spectrum of which showed no carbonyl band. The NMR spectrum (Fig.7) of this compound is

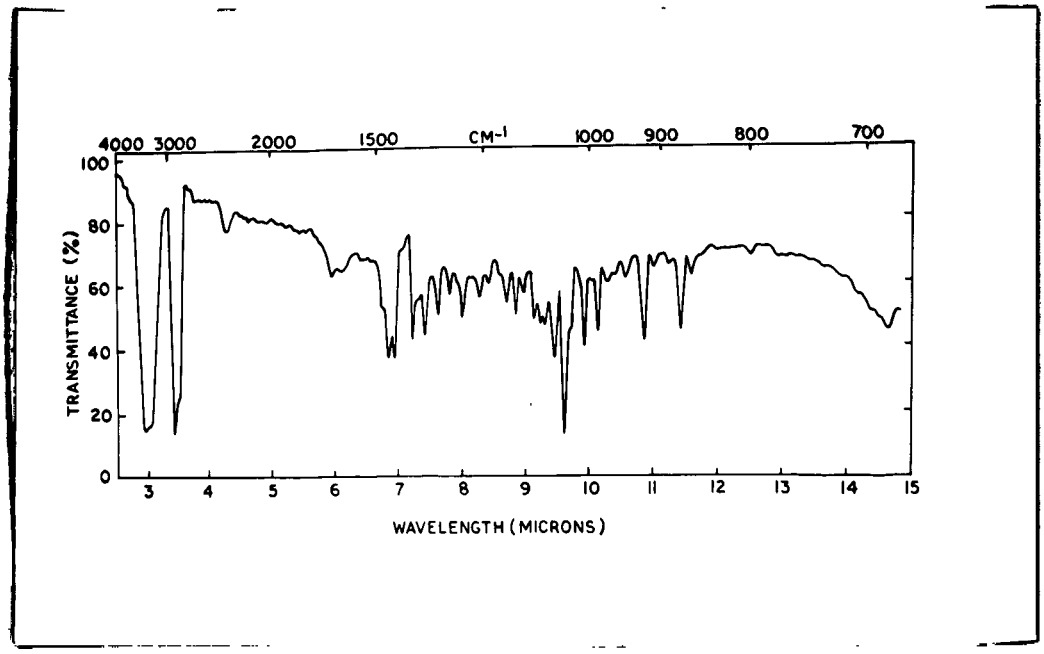


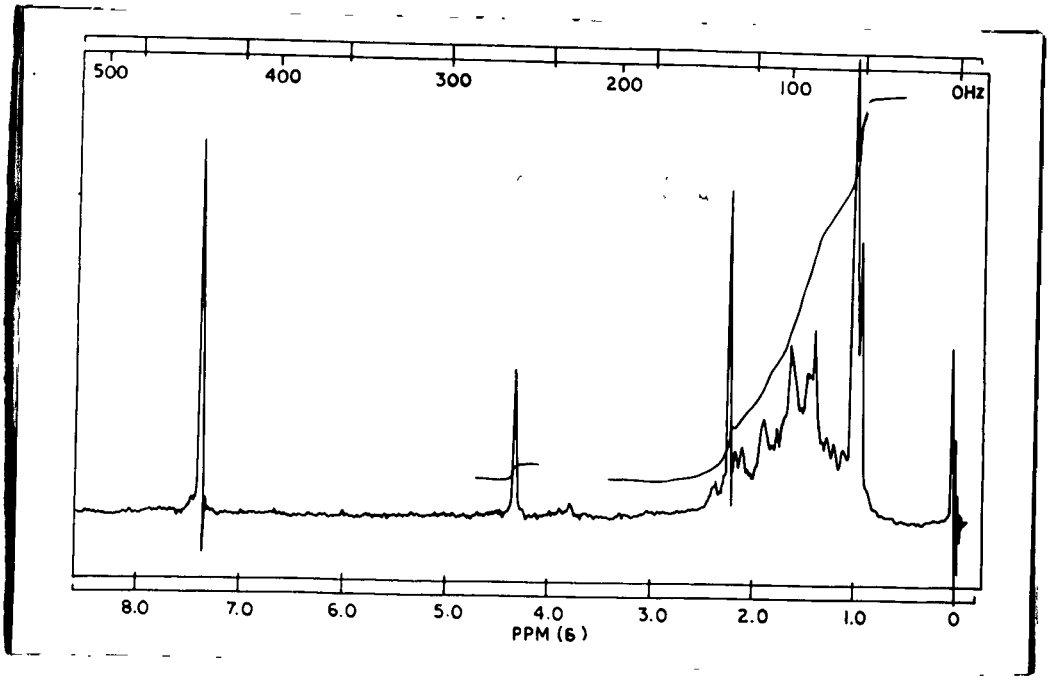
intriguing and can only be understood if it is assumed that the very strong singlet at 6.8 and the doublet downfield from this arise from an impurity, even though the product was homogeneous on TLC. The remaining signals in the spectrum can then be assigned to the methyl and methylene protons of a hydrocarbon, such as kaurane or phyllocladane, but absence of the secondary methyl doublet would still be inexplicable. Efforts to obtain a crystalline product from this reaction did not succeed. The above melting point is too high for kaurane and very close to that of phyllocladane but presence of impurities and failure to get a crystalline product do not allow a definite interpretation of the results of this reaction. It should be noted, however, that apart from extraneous impurities lack of homogeneity in the reaction product may also be due to the formation of the 16-epi compound e.g. 16-epiphyllocladane with α methyl, and β hydrogen at C-16.

Failure of these two reactions to provide a product of established structure and stereochemistry led to further investigation of the Huang Minlon reduction employing more drastic conditions than was done previously. Different procedure have been reported in literature for reduction of hindered ketones by this method (54). Thus the original procedure of Huang Minlon (55) was itself envolved to reduce 3-keto steroids in good yields which under the normal Wolff Kishner conditions afforded predominantly the 3-oxy-compounds. The reduction of C=O to CH-OH was attributed to the hydrolysis of the hydrazone intermediate during the reaction and subsequent

reduction of the ketone by methoxide. In the Huang Minlon variation this side reaction is avoided by taking an excess of hydrazine and distilling off hydrazine and water during the reaction. The application of the procedure was extended to 11-one steroids by Barton et al (56,57) who used anhydrous hydrazine and took precautions to exclude traces of moisture from the reaction. This procedure is, however, risky and as mentioned earlier, requires precautions which are not easily taken as anhydrous hydrazine is liable to explode at high temperatures.

The Huang Minlon reaction was therefore repeated employing the conditions used normally for 3-keto steroids which involve distillation of excess hydrazine and water till the reaction temperature rises to 195-200 and the reaction was continued for 8 hrs. TLC of the reaction mixture showed the formation of three products which were separated by column chromatography using the gradient elution technique. The major component of this reaction having m.p.171-172° displayed no carbonyl band in its IR spectrum (Fig.8) and was also not identical with the triol obtained earlier in this reaction. The NMR spectrum of the acetate of this compound m.p.135-40° (Fig.9) confirms the reduction of C=O to CH_2 since it shows no deshielded protons in the 3.5 to 3.8 region and the methyl resonances, no longer exposed to the deshielding effect of the carbonyl group, also appear at higher field (9.0, 6H, 9.04, 3H) compared to their position in CH-1 i.e. CH-2 acetate.


4000 3000 2000 1500 CM-1 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

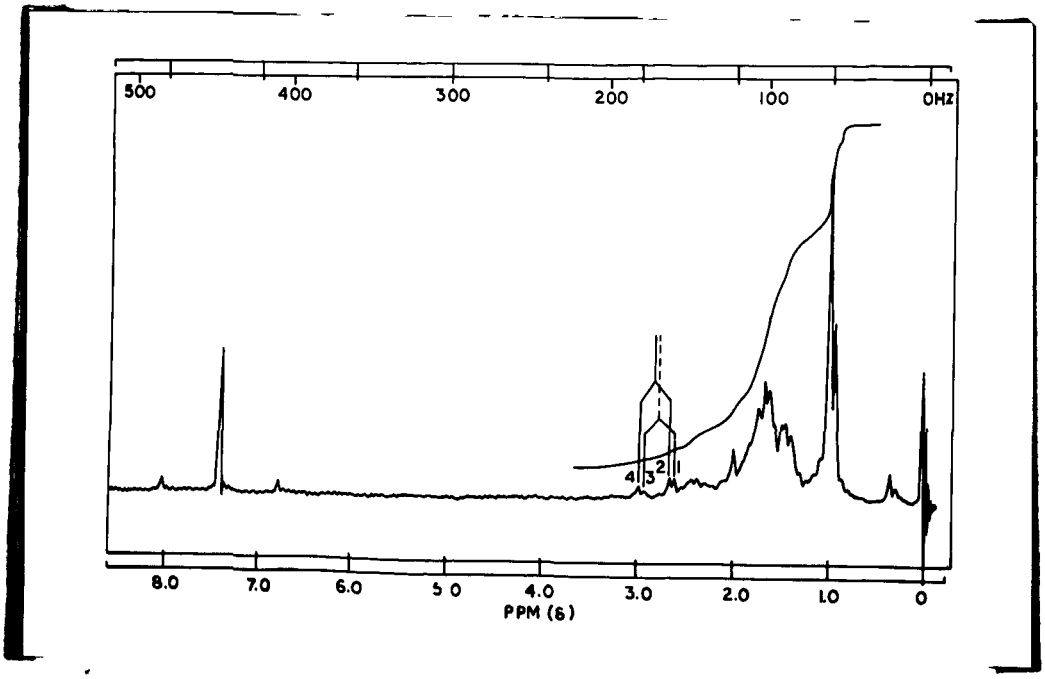

500 400 300 200 100 0Hz
8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0
PPM (δ)

The melting point of the Huang Minlon reduction product is 24° lower than that of kaurane 16.17 diol. The melting point of kaurane 16.17 diol monoacetate is not reported in literature but that of the 16.17 diol diacetate is 133°, which is very close to that of the monoacetate obtained above. This should have suggested that CH-2 is based on the phyllocladane skeleton but since phyllocladenol(7) is the only derivative of phyllocladane so far found to occur in nature the identity of the carbon skeleton of CH-2 with that of phyllocladane was overlooked at this stage of the work. The above results were sought to be explained instead by assuming that the diol was lower melting than the corresponding kaurane diol because of contamination by traces of some impurity. Repeated crystallisations, however, failed to bring about any change in the melting point which made it necessary to prepare one more derivative for comparison purposes. Consequently the diol was oxidised to the 17-norketone by treatment, as before, with periodate. This ketone did not crystallise readily from petroleum ether and other solvents were not found suitable either. Slow evaporation of petroleum ether ultimately gave crystals m.p. 95-100° which was improved to 98-100° by repeated crystallisation, in the same way, from petroleum ether. This melting point is again 24° lower than that of the corresponding dm kaurane derivative, 17-norkaurane-16-one. Though here also the difference in melting point was at first attributed to the presence of traces of impurities in

the periodate oxidation product, the possibility that the compounds obtained above were not kaurane derivatives had also to be considered.

The melting points of the corresponding phyllocladane derivatives were therefore looked up and were found to agree very closely with those of the above products. Thus the literature melting point of phyllocladane 16.17-diol is 172, that of its 17-monoacetate 136-37°, and of the 17-norketone 101° (11). These identifications were very kindly confirmed by Prof. R.C. Cambie through mixture melting point determination with authentic samples of phyllocladane 16.17-diol and the 17-monoacetates. Prof.Cambie also provided a sample of 17-nor-phyllocladane-16-one requested for and a mixed melting point determination of this with the product obtained from CM-2 also gave no depression.

In the NMR spectrum (Fig.10) of the norketone



two of the methyl resonances coincide at 9.0 and the resonance of the third methyl appears at 9.05. The only interesting feature of this spectrum is the AB pattern resulting from coupling (J_{gem} = 19 cps) methylene of the protons adjacent to the carbonyl. The chemical shifts of these protons (7.18 and 7.20) are given by the midpoints of lines 1,3 and 2,4.

CH-2 is thus conclusively shown not to have the kaurane nucleus, suggested by A.Chatterjee et al (58) for calliterpenone, in a paper on the diterpene constituents of Callicarpa macrophylla which appeared while this work was in progress. Calliterpenone has the same molecular formula, functional groups and spectral characteristics as CH-2 but since the plant material used in this study was collected from a different region a remote possibility remained that the two compounds were not identical. This was disproved by comparison of CH-2 with a sample of calliterpenone kindly provided by Prof.A.Chatterjee which showed the two compounds to be identical.

The designation CH-2 can therefore be dropped and the name given to the product by Chatterjee et al is used in the discussion hereinafter.

In view of the fundamental difference in the structural assignment, e.g. failure of the Huang Minlon reduction to reduce the cyclohexanone carbonyl and the discrepency in the results of some experiments, it is not necessary to go further into the details of the work reported by A.Chatterjee et al. The structure assigned by them differs not only in the stereochemistry of the nucleus

but also in the position assigned to the carbonyl group.

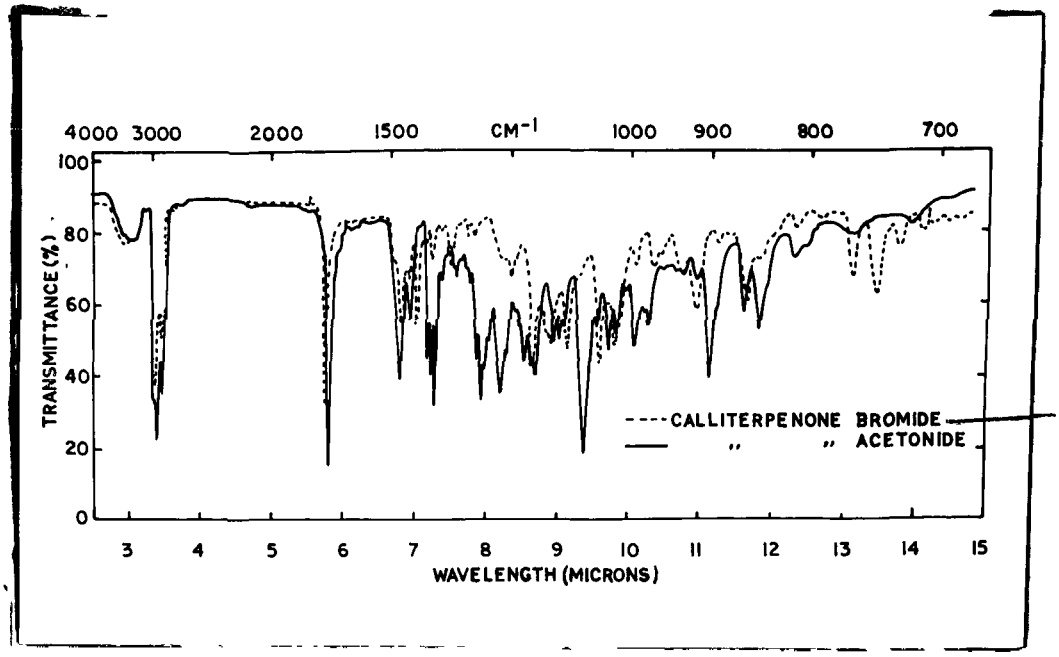
With the establishment of the phyllocladane carbon skeleton for callitarpenone (= CM-2) complete structural elucidation required only the location of the C=O function. Since only a limited amount of material was available a degradative proof of this feature was not attempted. Instead efforts were directed towards preparation of derivatives the NMR spectra of which could provide the necessary information for locating the carbonyl group. Since callitarpenone gives a positive Zimmerman test, it must have atleast one methylene adjacent to the carbonyl and the derived α bromoketone seemed the most appropriate compound for obtaining information on the environment of the carbonyl group.

N-bromosuccinimide was used initially for bromination and since the hydroxyls could have interfered, the reaction was applied first to the diketone obtained by periodate oxidation and later to the acetonide.

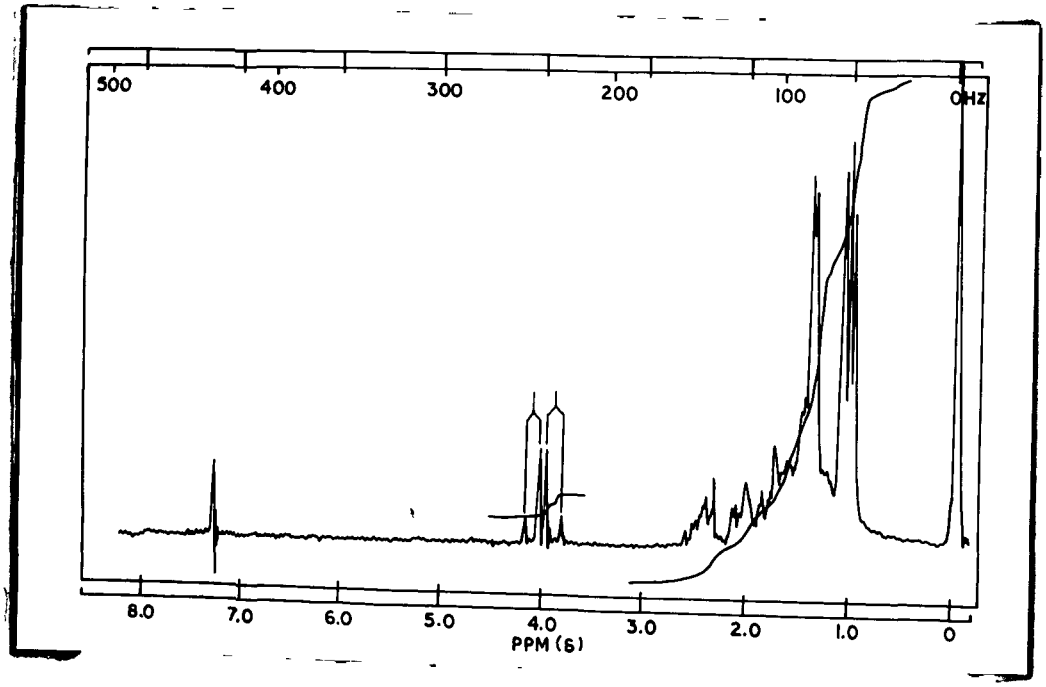
α-Halogenation of ketones with NBS, specially in cyclic systems, is not a very clean reaction and is complicated by dehydrohalogenation followed by allylic halogenation. The reaction is successful only if the reaction period is kept short ([58a]). Here also it was found that when the reaction was continued for 1/2 - 1 hr, TLC of the oil obtained on removal of the solvent showed it to be a mixture of 6-7 components but when the reaction was stopped after 5 min, TLC showed the formation of only 2 products. In neither case, however, could a crystalline product be obtained.

Bromine in acetic acid is normally used for α-bromination of ketones (59) but since the reaction was to be applied to calliterpenone acetonide there was some chance of hydrolysis of the acetonide under the acidic reaction conditions. Trimethyl phenyl ammonium tribromide has also been used for this reaction but in an analogous case (60) it afforded only an oily derivatives which must have been due to the presence of other products in the reaction mixture as bromides of such compounds are as a rule, nicely crystalline compounds. Consequently bromination with bromine-ether complex which seemed the mildest possible brominating agent, was attempted.

The acetonide used in this reaction was prepared by treatment of calliterpenone with acetone in presence of HCl. The residue obtained on evaporation of acetone was crystallised from petroleum ether. The melting point of the product 164-166° is the same as that reported by A.Chatterjee *et al* but TLC showed that it contained traces of an impurity. This was removed by column chromatography and the purified acetonide then melted at 175°. Its nature as acetonide follows from its IR and NMR spectra. The IR spectrum (Fig.11) is devoid of the prominent OH stretching band present in the spectrum (Fig.1) of calliterpenone, the slight absorption at 3400 cm^{-1} can arise from traces of moisture. The NMR spectrum (Fig.12) shows the C-17 methylene protons as a pair of AB doublets (J_{gem} = 9 cps) at 5.93 and 6.13 and the acetonide methyls at 8.63 and 8.66.



Calliterpenone bromoacetonide

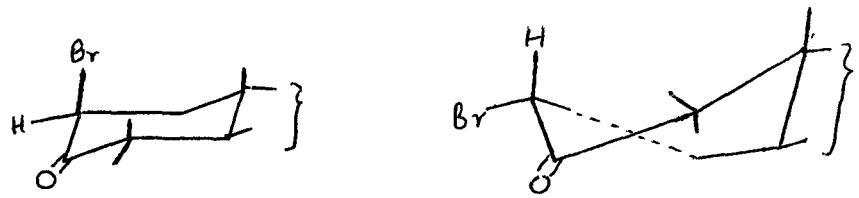


The bromine ether-complex was prepared according to the method given in literature (61). The complex was added to an ether solution of the acetonide till the yellow colour persisted for some time. The procedure was modified from that reported only to the extent that HBr formed in the reaction was washed off with dil. $NaHCO_3$ solution before the ether was evaporated. A solid began to appear even before all the ether had evaporated. Crystallisation from benzene-petroleum ether gave the pure monobromide though the yield was extremely poor (15 mg from 100 mg).

The IR spectra of α-brominated ketosteroids were studied by R.N.Jones et al (62). In the case of 3-ketosteroids it was found that α-monobromination resulted in a hypsochromic shift of the carbonyl band by about 13-19 cm^{-1} and a 25% reduction in its intensity. Dibromination to give the gem dibromide did not cause any further shift or change in intensity of the carbonyl band α,α' dibromination, however, brought about a further hypsochromic shift by about 20 cm^{-1} and a further decrease in intensity of the band. Another interesting feature of the IR spectra of steroid bromoketones is that the 11-β-bromo-12-ketosteroids show two bands at 1730 (s) and 1706 (w) cm^{-1} in the carbonyl region of the spectrum.

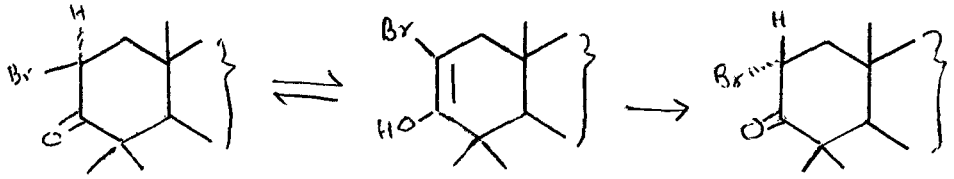
The above hypsochromic shift in the carbonyl stretching frequency occurs because of the coplanarity of the $\overset{+}{C}{-}\overset{-}{O}$ and $\overset{+}{C}{-}\overset{-}{Br}$ dipoles which enhances the double bond character of the carbon oxygen bond by suppressing the

contribution of the $>\overset{+}{C} - \overset{-}{O}$ resonance structure. As such one would expect that in 3-ketosteroids there should be no hypsochromic shift in the case of the 2-βbromo derivative where the bromine atom being axial the $\overset{+}{C} - \overset{-}{Br}$ duplo is not coplanar with the carbonyl dipole. This was very surprisingly found not to be the case and of the two bromides the 2-β-bromoc compound was found in fact to absorb at higher frequencies 732 cm^{-1} (Δcm^{-1} = 9) than the 2α-isomer, 1726 cm^{-1} (Δcm^{-1}= 4). This anomaly was explained by Barton et al (63) who suggested that the unfavourable interations of the axial bromine in 2-β bromosteroids cause ring A to assume the best conformation in which the $\overset{+}{C}=\overset{-}{O}$, $\overset{+}{C}-\overset{-}{Br}$ dipoles are again coplanar, the bromine having acquired the equatorial configuration in the change of conformation.



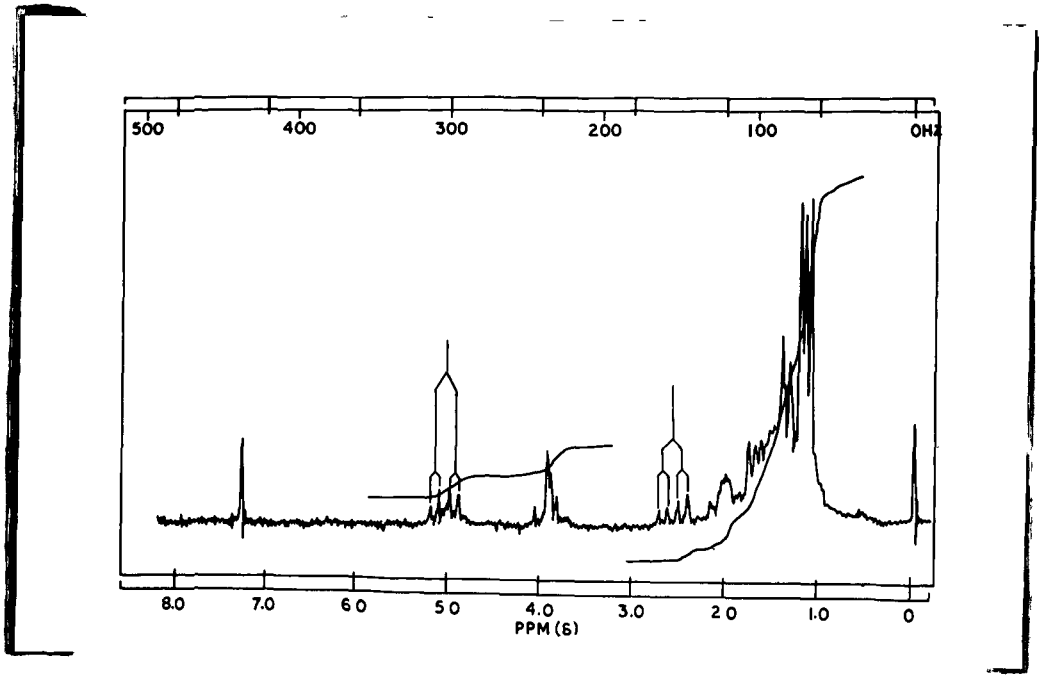
To return to the IR spectrum (Fig.11) of the bromo derivative of calliterpenone acetonide the C=O stretching frequency is seen by comparison with that of the acetonide to have undergone a shift of 20 cm^{-1} and to occur at 1740 instead of 1720 cm^{-1} as in the acetonide.

Since bromination under the conditions used involves Br_2 attack on the enol, bromine should initially be β in the case of a 3-keto compound but should change over very rapidly to the α configuration by way of the enol (LXXXVI).



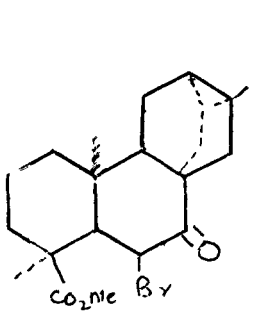
LXXXVI

The NMR spectrum of the bromide (Fig.13) shows distinctly the proton (H_X), geminal to bromine, as a quartet centred at 4.96. This quartet results from the coupling of (H_X) to two neighbouring protons. H_A and H_M so as to give an AMX pattern with J_{AX} = 14 cps and J_{MX} = 6 cps. The larger coupling constant (J_{AX} = 14 cps) requires a diaxial disposition of the protons H_X and H_A. The quartet of the

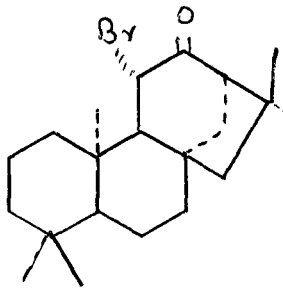


third proton H_M is discernible just outside the methylene envelop. Its coupling constants are found to be J_{AM} = 13 cps and J_{MX} = 6 cps J_{AM} corresponds to geminal coupling and the value is consequently large.

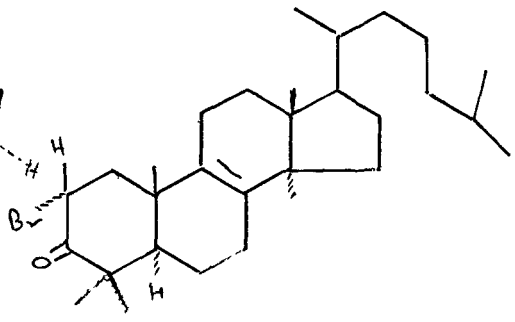
In the phyllocladane nucleus this situation is possible only with the carbonyl at C-3 or C-1 with equatorial bromine at C-2. Further support for this interpretation is provided by comparison with the spectrum of 2α-bromocholestane-3-one (64). The spectra of the two compounds are strikingly similar in the regions concerned with the quartet of the C$\underline{H}$Br proton at 5.20 (J_{AX} = 13 cps, J_{MX} = 6 cps) and the quartet of the equatorial C-1 proton at 7.35 (J_{gem} = 12 cps, J_{MX} = 6 cps). Thus there is a difference (0.24 ppm) in the chemical shift of the C$\underline{H}$Br protons in the two compounds. This difference is small when compared to the difference with the chemical shifts, 5.5 and 4.35, of the C$\underline{H}$Br proton in (LXXXVII) (65) and (LXXXVIII) (66) respectively. The chemical shift reported for the C$\underline{H}$Br proton in 2α-bromo lanost-8-ene-3-one (LXXXIX) (67) is 4.88 and in 2-α-bromo beyrol-3-one (XC) (68) the mid point of the equal hight signals reported lies at 4.86.



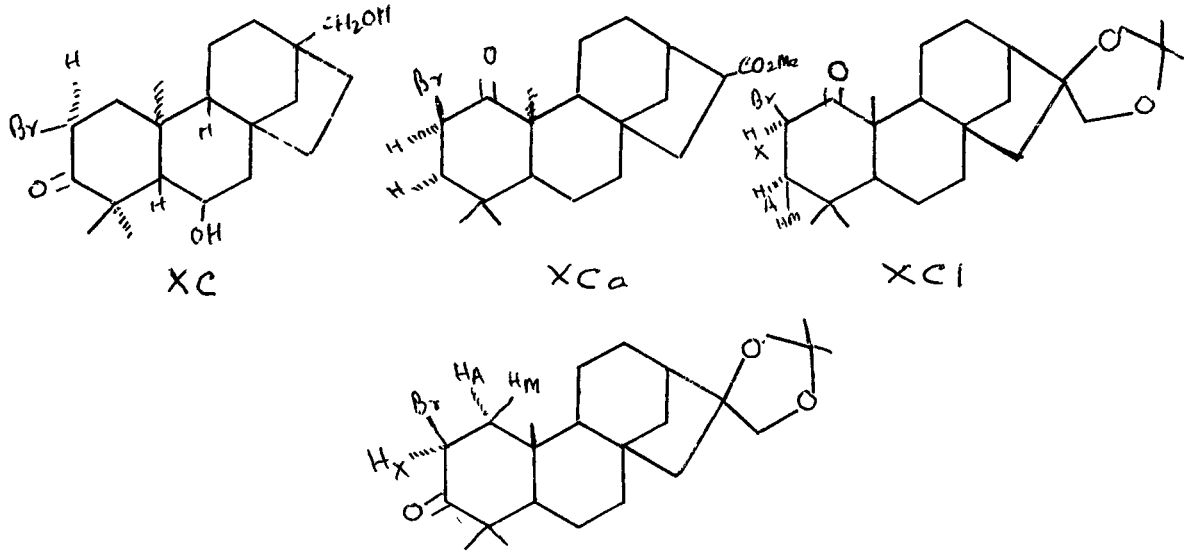
LXXXVII



LXXXVIII



LXXXIX



XC XCa XCI

XCII

As against this the quartet of the C$_{11}$Br proton in (XCa) (69) occurs at 4.44. These comparisons therefore support a 2-α-bromo-3-keto structure for the bromide.

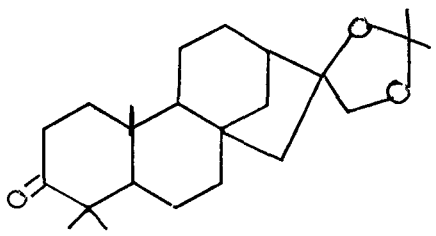
The bromoketone is thus shown to have characteristics compatible with only two structures (XCI) and (XCII). (XCI) should be preferred both from biogenetic consideration and the fact that with the exception of compounds eg. (XCa) columbin (70) ... no other C-1 oxygenated diterpene is known.

The other notable feature of the NMR spectrum is the perturbation of the C-17 methylene resonance, the AB doublets of which are not as distinct as they are in the spectrum of the acetonide. There is, further, a downfield

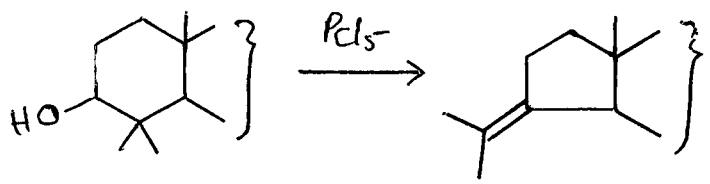
shift of the methyl resonances which can also be ascribed to the deshielding effect of bromine. The possible use of this in the assignment of the position of the carbonyl group will be discussed later.

Of the methods available for deciding between the two structures (XCI) and (XCII) for calliterpenone the one based on Baeyer Villiger oxidation appeared preferable. This method was used in assigning the position of the carbonyl in abbeokutone (71) but the very poor yield reported was discouraging. However, the other possible approach usual in triterpenes, rearrangement of the derived alcohol (72) (XCIII) to (XCIV) has not been applied to diterpenes for reasons which are not apparent. A test experiment showed that the reaction of the acetonide with P_2O_5 in benzene afforded an oil found by TLC to be a mixture.

The acetonide was therefore oxidised with perbenzoic acid, which can be readily prepared according to the reported procedure (73). TLC check up showed that the reaction was extremely slow but afforded only a single product. It was therefore allowed to continue for 5 days but even then only partial conversion of the starting material (XCV) was observed. Since no further increase in the intensity of the spot of the reaction product was



XCV

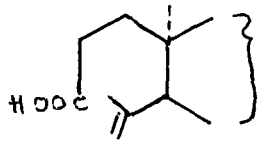


XCIII XCIV

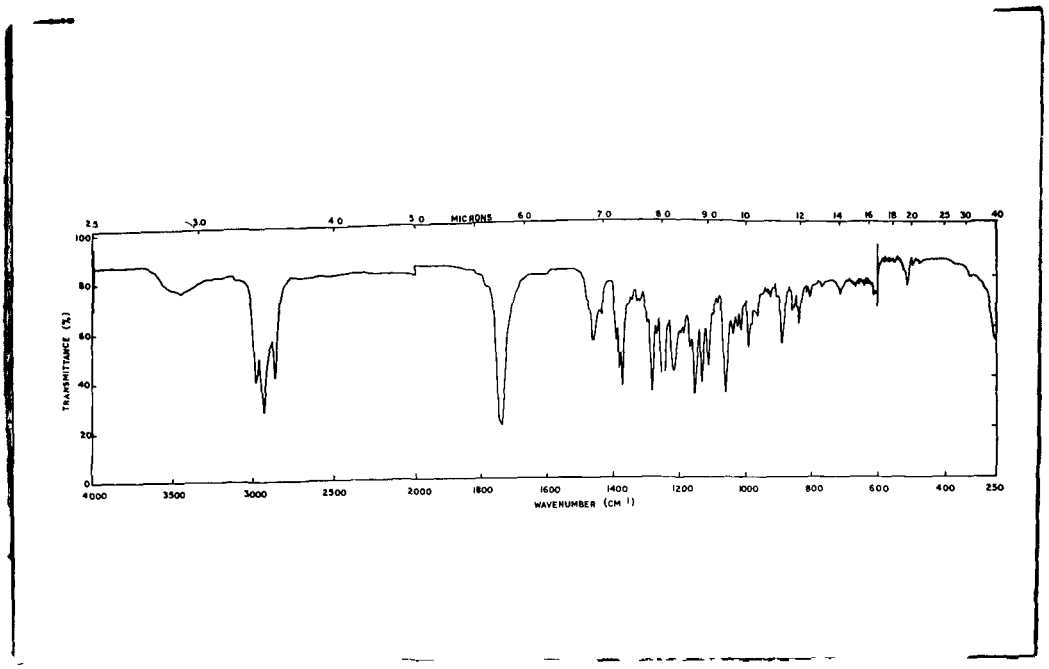
evident the reaction was stopped by addition of a few drops of dimethyl sulphide. This work up procedure was preferred to the conventional one involving ferrous sulphate extraction as the latter gives highly coloured solutions and the product is difficult to purify. Extraction with aqeous Na_2SO_3 results in emulsion formation and the work up is needlessly complicated. Evaporation of $CHCl_3$ and DMSO formed by sulphide oxidation left a solid which was shown by TLC to be a mixture of the starting material and the reaction product. Crystallisation from petroleum ether-benzene failed to bring about a separation and resolution was therefore effected by chromatography over silica gel. The amount of the reaction product available after this separation turned out to be much less than anticipated at first on the basis of TLC. The product was however nicely crystalline and one crystallisation from petroleum ether benzene afforded a pure compound as needles m.p. 164°. The amount was just sufficient for the NMR spectrum but this was conclusive.

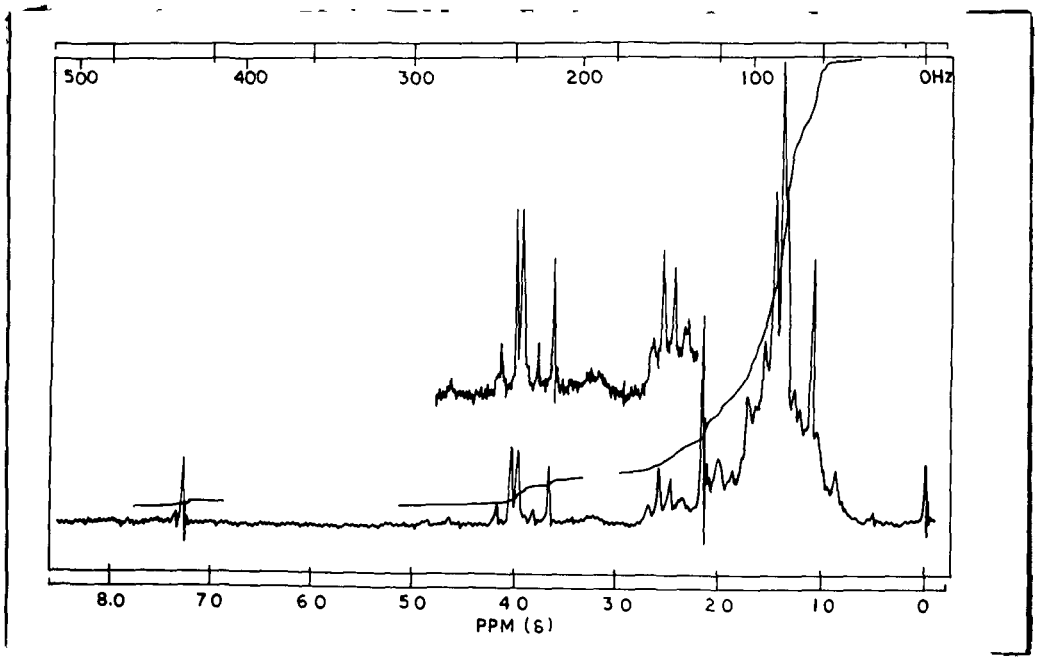
In abbeokutone where m-chloroperbenzoic acid was used the product of this reaction was the unsaturated acid (XCVI). The NMR spectrum Fig.14) of the product m.p.164° however showed no olefinic protons.

The IR spectrum (Fig.15) shows the carbonyl band at 1740 cm^{-1} indicating formation of a lactone the structure of which follows from its NMR spectrum. The NMR spectrum differs most strikingly from the spectrum of the



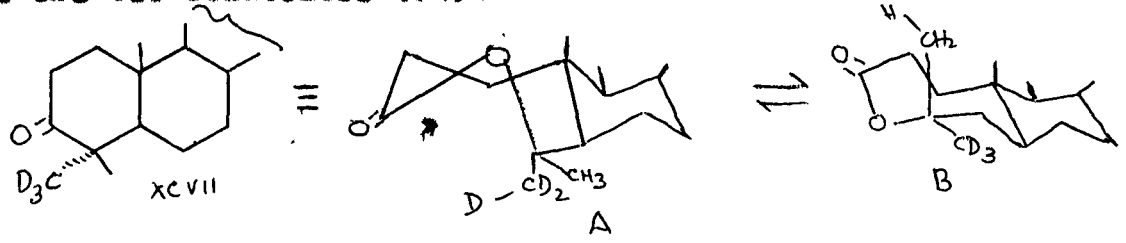
XCVI

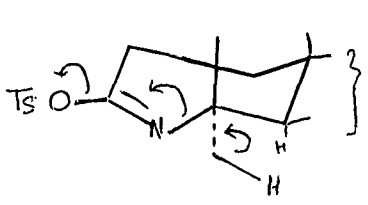

MICRONS
TRANSMITTANCE (%)
WAVENUMBER (CM⁻¹)


500
400
300
200
100
0HZ
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ)

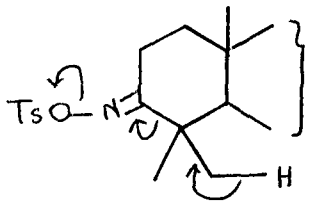
acetonide in the position of the tertiary methyl signals two of which were shifted downfield to δ.63 and δ.66 so as to overlap partially with the singlets of the gem dimethyl groups of the acetonide moiety which give rise to resonances at δ.55 and δ.63. The singlet of the third tertiary methyl is at δ.93 which is approximately the same value as that of one of the tertiary methyl singlets in the acetonide (Fig.12) These results are compatible with only one structure (XCII) as in the alternate formulation (XCI) only the C-10 methyl would have been deshielded.

Since Baeyer Villiger oxidation of 3-keto-terpenoids normally yields lactones the production of (XCVI) from abbeokutone is not understandable. It is likely that traces of mineral acid in the commercial m-chloroperbenzoic acid led to this result. The unsaturated acids corresponding to (XCVI) are usually obtained by pyrolysis of the lactone and the reaction has been shown to be non stereospecific since hydrogen abstraction takes place from both the axial and the equatorial C-4 methyls. This lack of stereospecificity has been attributed to the existence of the 7-membered heterocyclic ring in two different conformation A & B so that if in the starting material for the Baeyer Villiger oxidation (XCVII) the labelled methyl is equatorial it will be axial in conformation A, and equatorial in conformation B of the oxidation product. The actual proportion of the label eliminated should therefore depend on the relative population of the two conformers (74).





XCVIII



XCIX

As against this the abnormal Beckman rearrangement, which has been utilised for such biogenetically significant studies, has been found to be stereospecific. The suggested mechanisms (75) and (76) involve ring opening either of the amide (XCVIII) or the oxime (XCIX).

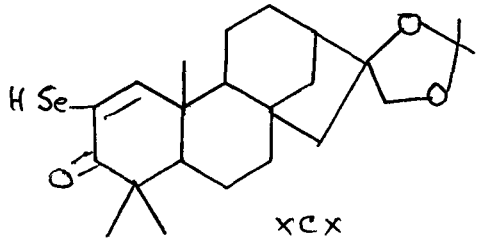
In one of the earlier experiments an attempt was made to obtain an α,β-unsaturated ketone from the acetonide (XCV). Since N-bromosuccinimide was found to give mixtures, SeO_2 was used for the purpose. This reagent normally introduces a carbonyl group α to an existing carbonyl function, which enolises to give a diosphenol if an adjacent hydrogen is available.

When the acetonide (XCV) was heated for some time with a suspension of SeO_2 in moist benzene a crystalline product was obtained after extensive purification by chromatography and crystallisation. The melting point of this product, which was soluble in petroleum ether-benzene was abnormally high and indistinct. It formed hard brick

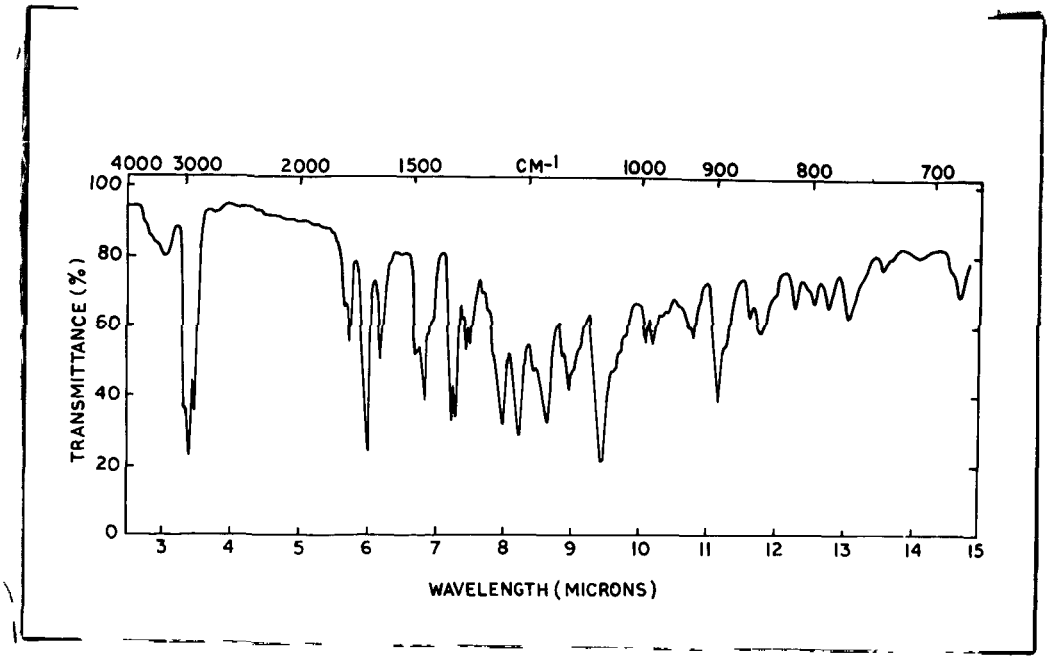
like crystals which softened at about 230 and melted indefinitely. TLC however showed the product to be pure and it was therefore analysed. The analytical values were very unexpected for a compound of this series and suggested incorporation of selenium. The mass spectrum also showed M^+ at 438 corresponding to the molecular formula $C_{23}H_{34}O_3Se$.

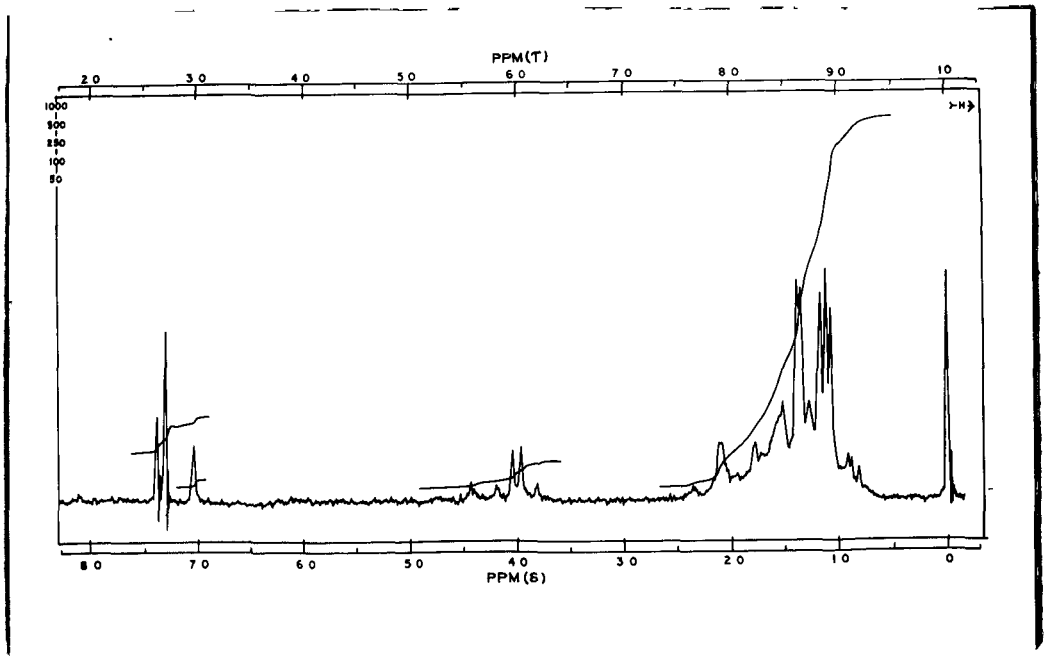
From its spectral data this compound can be formulated as (XCX). Thus the IR spectrum (Fig.16) shows bands at 1675, 1760 and 1770 cm^{-1}. The first of these can be assigned to ν C=O and the second to SeH. The value reported for ν Se=C in literature (77) is 962 and for ν SeH 2310 cm^{-1}. The difference of the former from the observed value 1760 is too large and the C=Se function must therefore be assumed to be present in the equivalent of the enolic form. The shift of 400 cm^{-1} from the literature value can be attributed to hydrogen bonding with the adjacent carbonyl. The UV spectrum is exactly analogous to that of diophenol showing a prominent band at 260 nm. The NMR spectrum (Fig.17) shows the singlet of the proton of the double bond at 2.6 and, apart from the signals common to the acetonide, only one more singlet at 2.94 which can be attributed to the SeH proton.

A compound of this type has not been reported in literature although formation of selenium containing products of undefined structure has been observed.

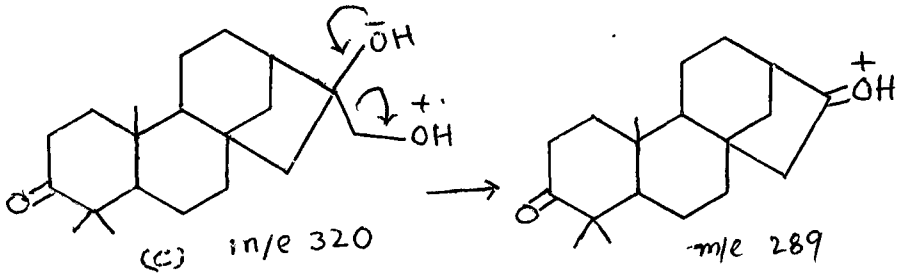


XCX

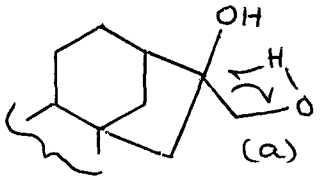

4000 3000 2000 1500 CM-1 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)


PPM(T)
2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10
8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0
PPM(δ)

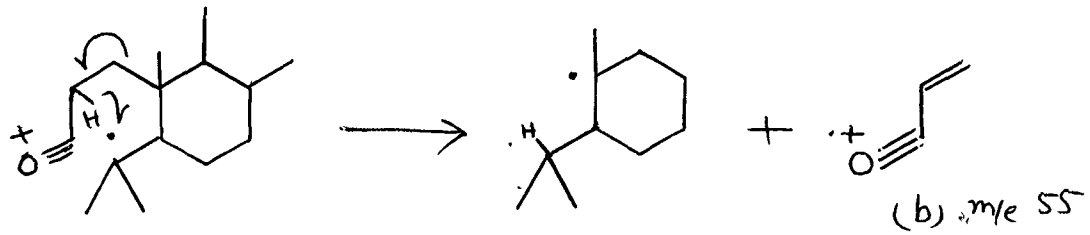
The mass spectrum of calliterpenone(c) showed M^+ at 320. The base peak at 289 arises from the loss of the CH_2OH fragment as shown.



The peak at 290 might arise thereby a hydrogen transfer reaction involving a four centre square planar



transition state (a). No such explanation can be advanced with certainty for most of the other peaks in the mass spectrum given on the next page. Thus the ion (b) formed as shown could be considered responsible for the peak at m/e 55 but this can not be true since a peak of comparable intensity at this value is observed also



in the spectrum of the diol (LXI) which does not have an oxygen at C-3.

Calliterpenone

m/e	%	m/e	%	m/e	%
41	99	95	48	149	14
43	66	105	31	151	16
53	20	107	42	159	23
55	61	109	42	216	17
67	54	119	30	230	10
69	44	123	23	247	19
77	24	125	22.5	271	21
79	46	133	24	289	100
81	54	137	24	290	33
91	46	145	19	302	7
93	46	147	21	320	4

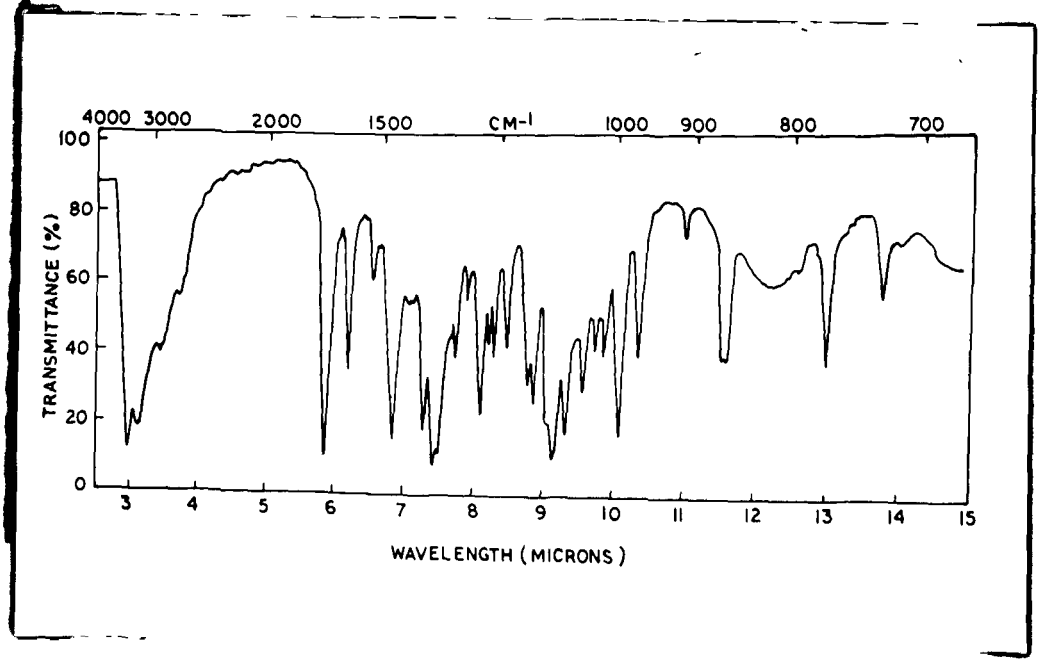
The mass spectrum of the acetate is similarly unhelpful from the structural point of view and offers only evidence of the formation of a monoacetate by M^+ at m/e 362.

Flueggea microcarpa (Euphorbiaceae)

The plant known locally as Bainc, was collected from Sahasradhara near Dehra Dun. According to literature it is applied externally to wounds and is also a fish poison (78).

A compound molecular formula $C_{14}H_{16}O_9 \cdot H_2O$ was obtained from the alcohol extract of the plant it was soluble in water, alcohol and acetone but insoluble in all other organic solvents which suggested its glycosidic nature. It could not however be hydrolysed under conditions normally sufficient for cleavage of the glycosidic linkage which made identification as glycoside somewhat doubtful.

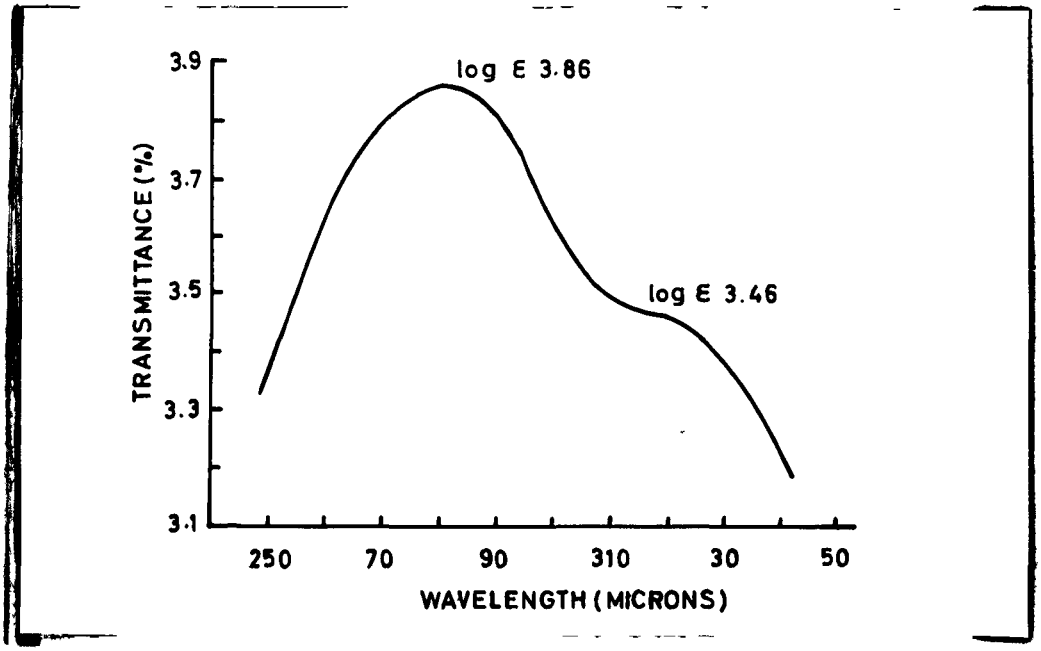
The compound did not give the specific colour reaction of flavonoids but its alkaline solution had the bright yellow colour characteristic of coumarins. The IR spectrum (Fig.18) supported the conclusion showing C=O



1725 •C•O•C at 1250. The spectrum further showed strong hydroxylic absorption between 3400 - 2900 cm^{-1} and aromatic bands at 1660,1545,770,730 cm^{-1}. That some of the hydroxyls are phenolic was shown by the green ferric colouration.

The UV spectrum (Fig.19) shows strong absorption at 280 nm (log ε 3.8) and an inflection at 322 (log ε 3.46) which is more in accord with an isoflavone or flavone nucleus than a coumarin one.

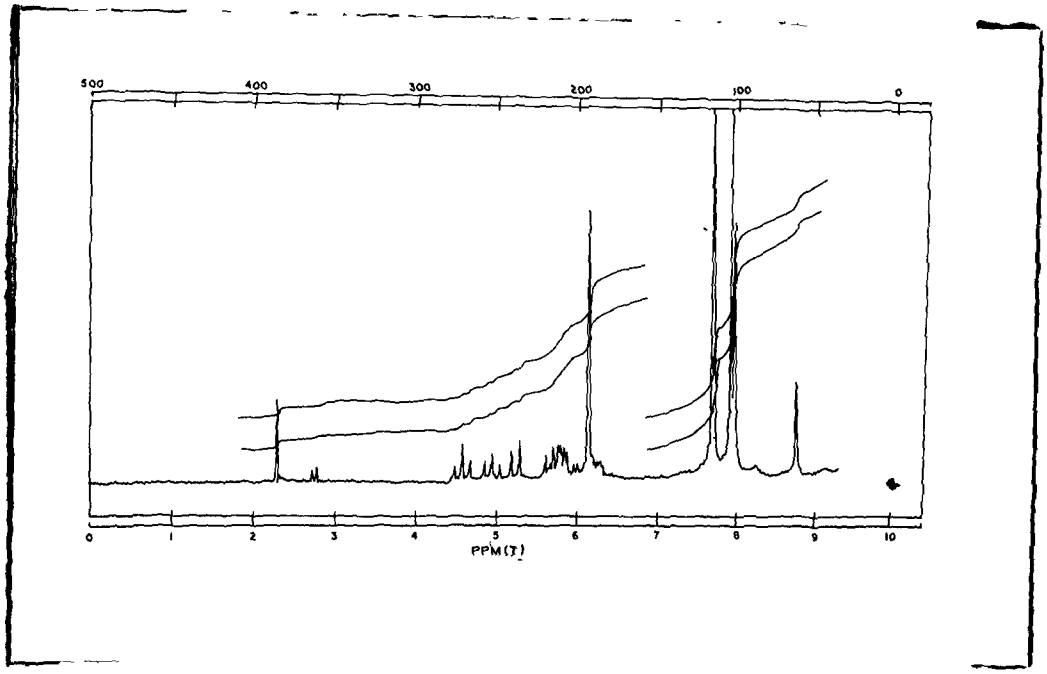
The NMR spectrum of the compound was also not in accord with the suspected presence of the coumarin nucleus, atleast of the more commonly encountered type since it lacked the doublets of the olefinic protons of the heterocyclic ring. It showed only one sharp singlet in the aromatic region, the midfield region showed presence of several unresolved signals projecting above a broad envelop.



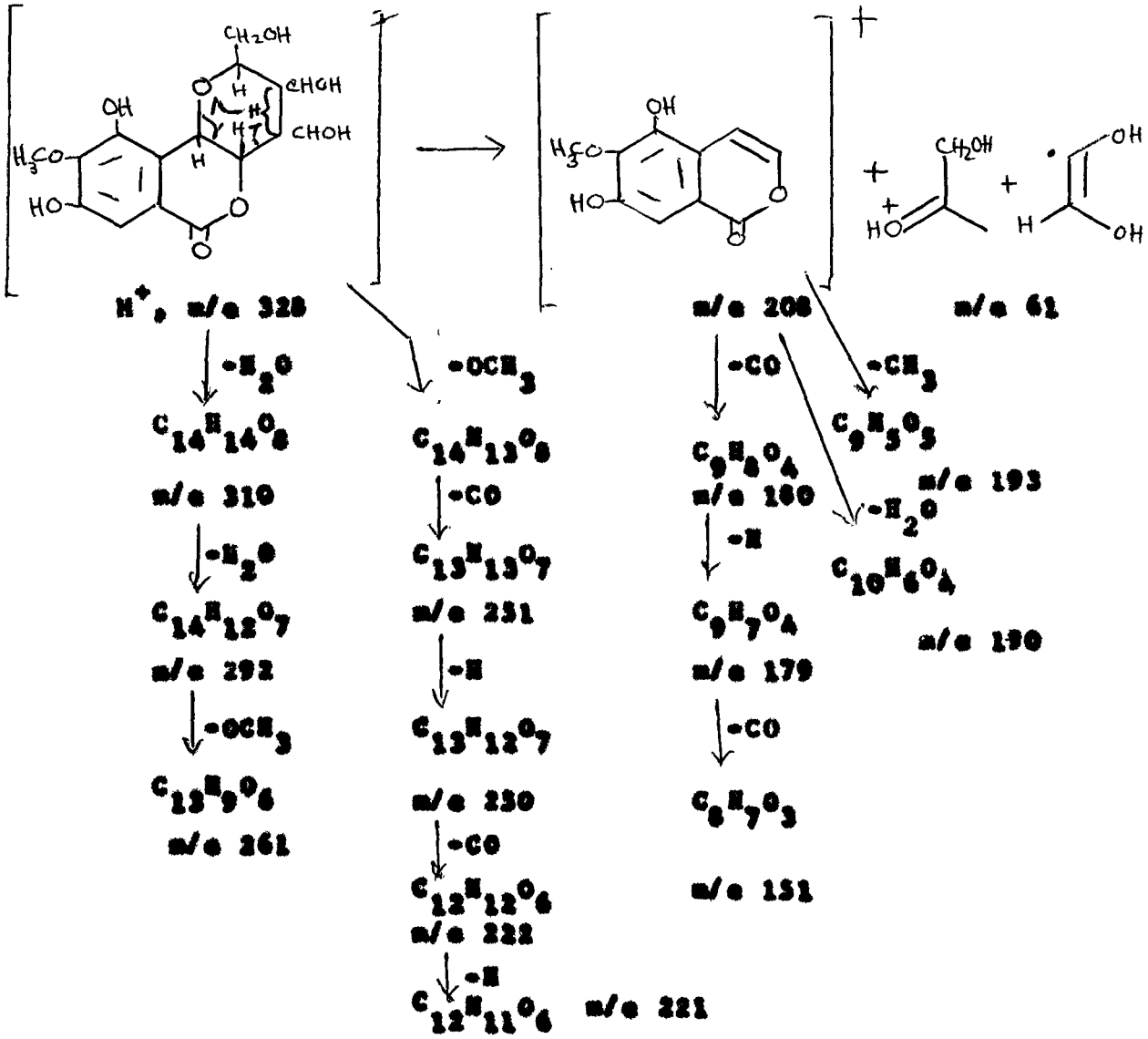
This made certain of the identification of an glycoside but the nature of the compound remained uncertain beyond this.

Acetylation of the compound gave a penta acetate the NMR spectrum (Fig.20) of which was much more definitive. It showed the presence of two aromatic acetyl methyls and three aliphatic acetyl methyls by signals at 7.7 and 7.9, taking the integral of an aromatic methoxyl at 6.15 as reference for integration. The aromatic region of the spectrum on the same basis showed only one aromatic proton as singlet at 2.3 which established the presence of a penta substituted benzene ring. The glycosidic proton appears as an ABC pattern between 4.3 and 5.3, and 5.66 and 5.8. The signal of one more proton appears to washed by the methoxyl signal.

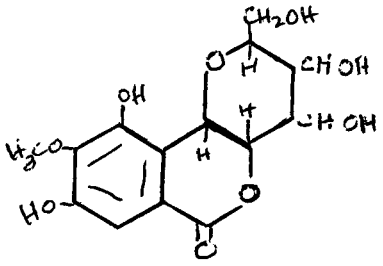
Since the compound could not be hydrolysed it could only be a C-glycoside. These features could be



accommodated on the assumption that the compound was bergenin, an isocoumarin C-glycoside with which the melting point of the compound and its acetate also agreed. Further support for this identification was provided by the mass spectrum which shows M^+ at 328 and fragment whose formation could be interpreted as shown below.



Final confirmation was obtained by mixed melting point determination with an authentic bergenin kindly provided by Prof.Walker.

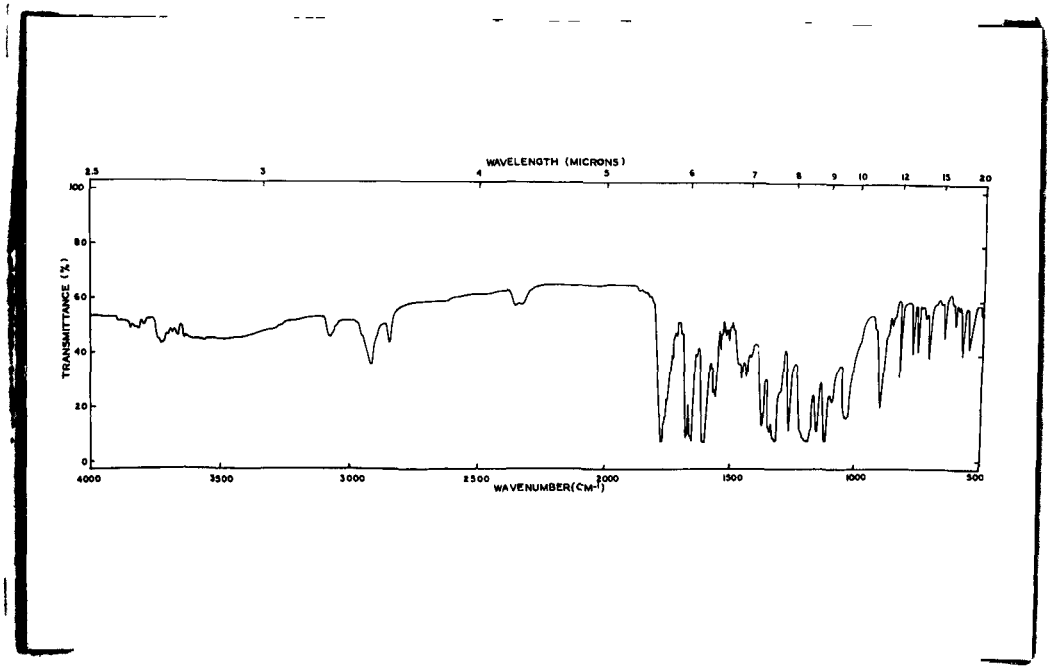


Bergenin

Rhamnus triquetra (Rhamnaceae)

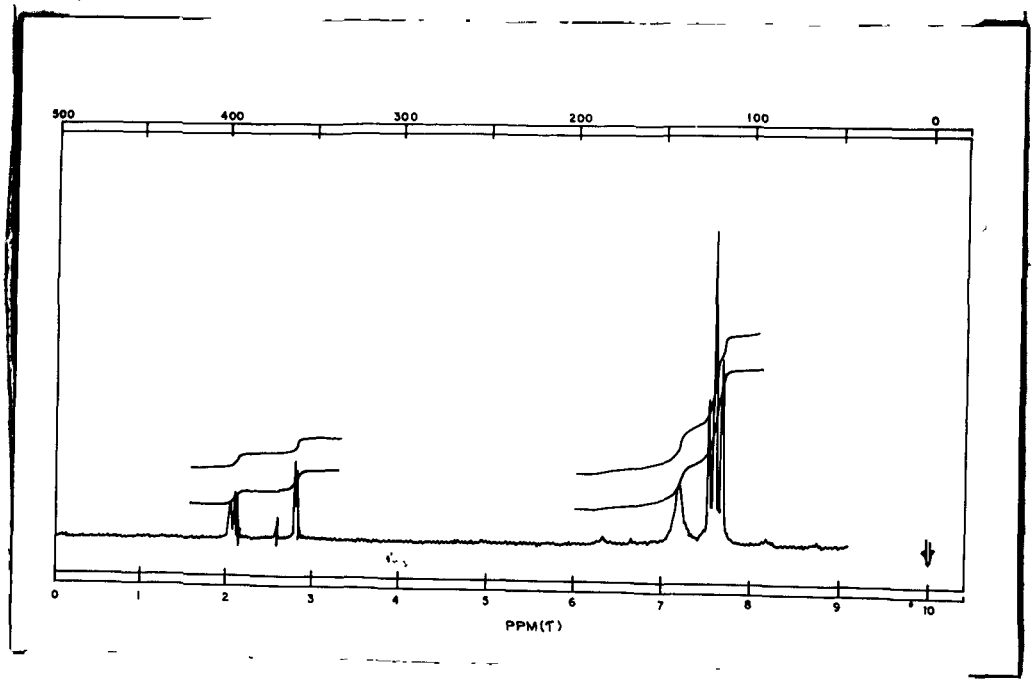
Like **Flueggea microcarpa** the bark of this plant which grows in hilly regions of north India is also reported to possess toxic properties (79).

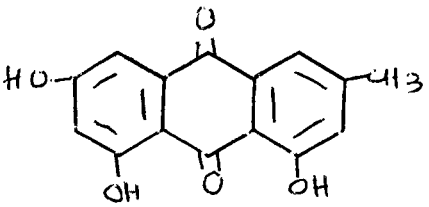
The ethanol extract of the plant afforded a compound shown by analysis and M^+ at m/e 270 to have the molecular formula $C_{15}H_{10}O_5$. The orange red colour high oxygen and low hydrogen content suggested an anthraquinone type of molecule. The presence of phenolic OH was shown by the light green ferric colouration. The IR spectra (Fig.21) showed OH at 3450 - 3200 and C=O at 1630 cm^{-1}. A broad band between 1600-1550 and a sharp one at 760 cm^{-1} provided evidence for the aromatic nature of the compound. Since the compound was not soluble in chloroform its NMR could not be measured.



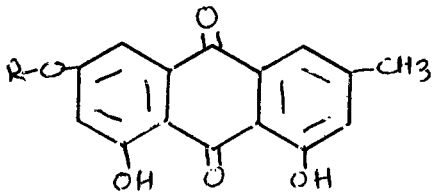
Acetylation gave a triacetate the NMR spectrum (Fig.22) of which showed three acetate methyls at 7.6, 7.7 (s,6H and s,3H) and the aromatic methyl at 7.5. The only other proton in the molecule resonate between 2.0 to 2.9.

On the assumption of an anthraquinone nucleus the two doublet at 2.6 and 2.4 (J=2 cps) can be assigned to the peri protons and the other pair of doublet at higher field to aromatic protons. The spectral data accords only with emodin with which the melting point of the original compound and its acetate also agrees. The other compound which was obtained by prolonged ethyl acetate extraction of the aqueous solution of the alcohol extract gave emodin on hydrolysis and its melting point agrees with that of frangulin. This was further confirmed by paper chromatographic identification of rhamnose.



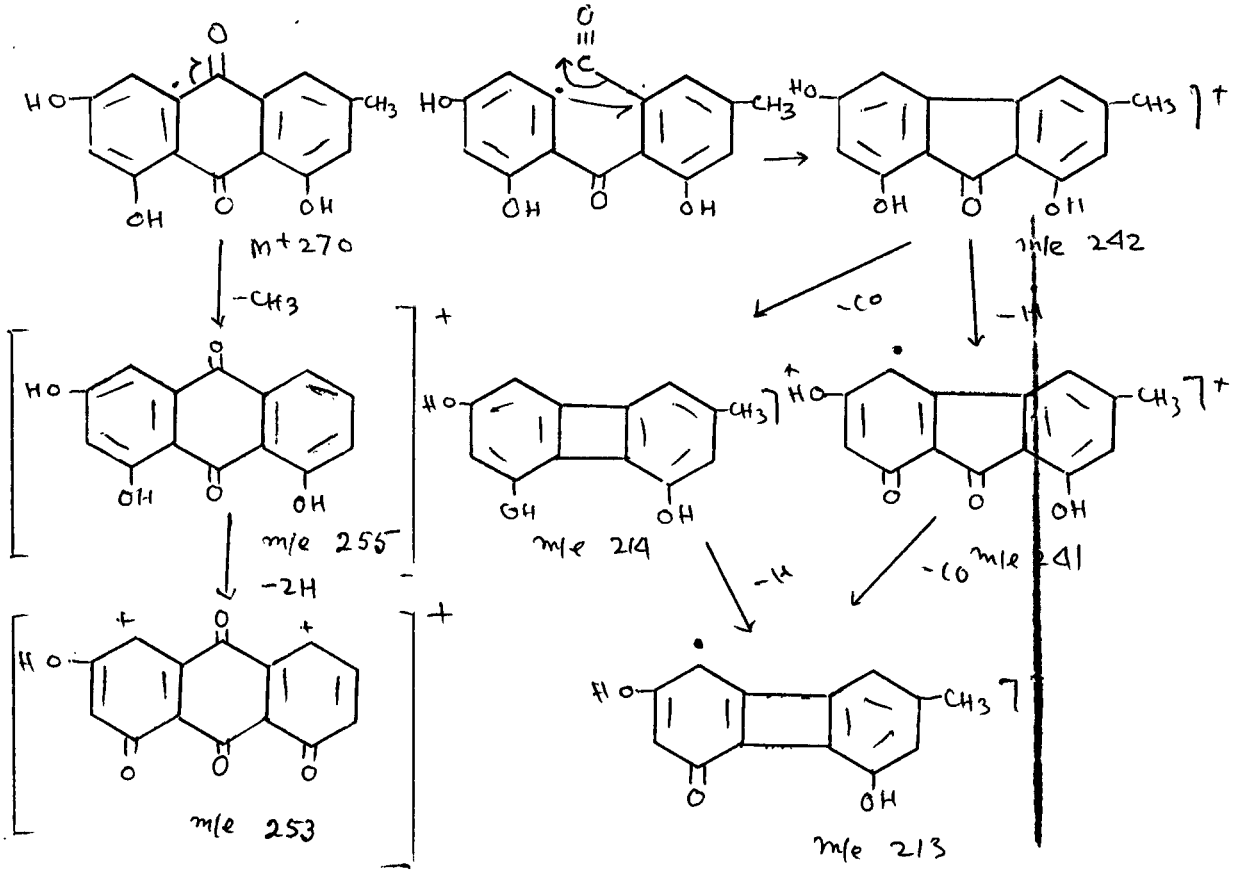


Emodin



Franqulin R = Rhamnose

The mass spectrum of quinone have been reported by J.H.Beynon et al (80) but are not extensively covered as those of flavenoids and coumarins. The major ions here are found by successive losses of CO as shown below.

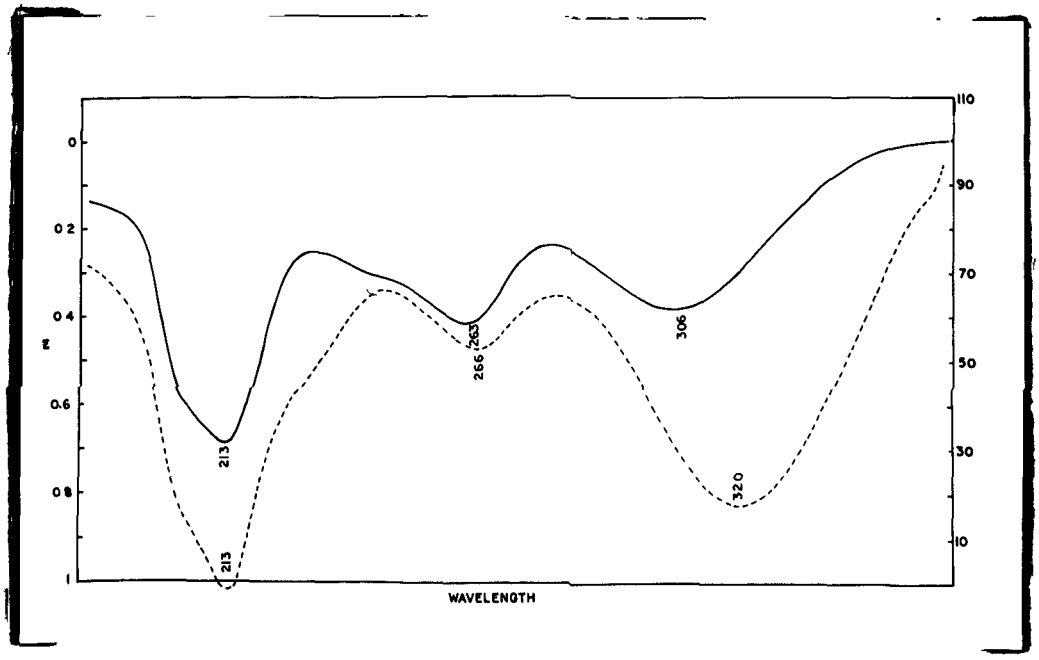


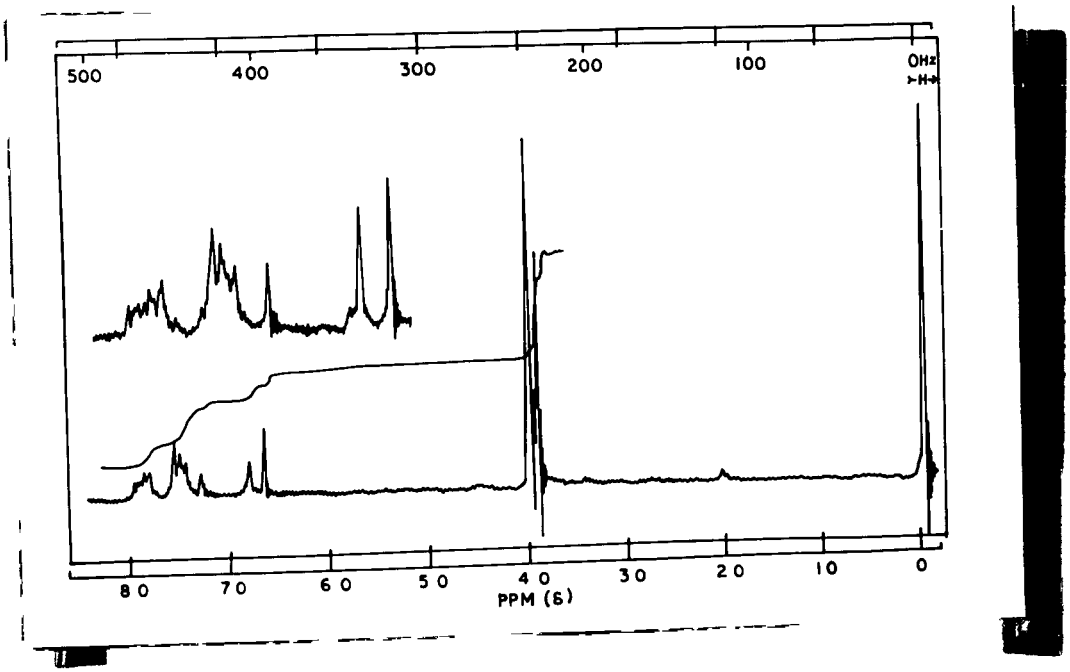
Colebrookia oppositifolia (Labiatae)

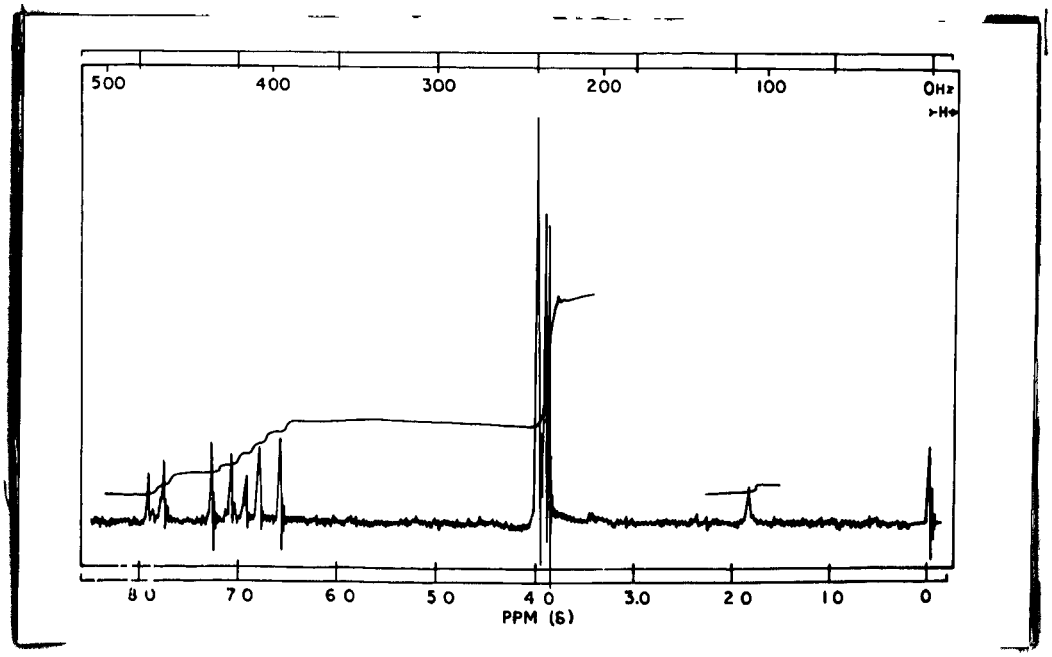
This is also a medicinal plant (91) and was collected like others from the Dehra Dun region.

The petroleum ether extract of the plant afforded on column chromatography two compounds m.p.165-67° and 140-42° both of which gave negative tests for flavones. Elemental analysis however disclosed the molecular formula $C_{18}H_{16}O_5$ and $C_{19}H_{18}O_6$ respectively. The elementry composition and the presence of three methoxyl in the first and four in the second of the above products suggested that both are C-15 compound and as such flavonoids. The UV spectrum (Fig.23) showed maxima at 263, 306 nm in the first and 266 and 320 nm in the second which is in agreement with the presence of a flavone nucleus.

The NMR spectrum (Fig.24 and 25) provided the information needed for structural assignment. The C-18




500
400
300
200
100
0Hz
H
80
70
60
50
40
30
20
10
0
PPM (δ)

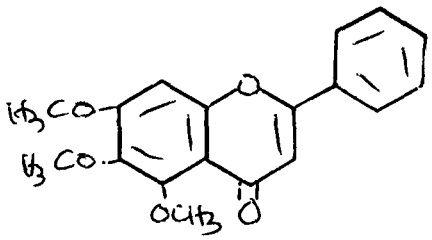

500
400
300
200
100
0Hz
H
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
PPM (δ)

compound singlets at 6.0 and 6.14 for 6 and 3 protons r· respectively in agreement with the presence of 3 methoxyls. The aromatic region of the spectrum shows two isolate protons at C-8 and C-3 by singlets at 3.2 and 3.28.

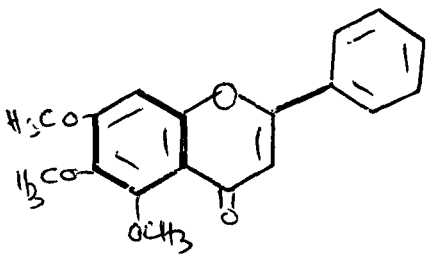
Protons of an unsubstituted ring B are responsible for the two multiplets at 2.5 and 2.1. The pattern is similar to the tectochrysin reported by Mabry (82). The compound is thus shown to have structure (A).

The C-19 compound shows a 6 proton singlet and two 3 proton singlets at 6.01, 6.09 and 6.12. There is thus 4 methyls in the compound one of which must be located at 4' so as to give rise A_2B_2 pattern for the 2'6' and 3'5' protons at 212, 2.22 and 2.95, 3.04 respectively. The remaining singlet at 3.20 and 3.42 can be assigned to proton at C-8 and C-3. This leads to structure (B) for the compound.

These compounds shows a very unusual substitution pattern and were reported very recently (83, 84, 85). The mixed m.p. determination with these compounds gave no depression.



(A)



(B)

EXPERIMENTAL

EXPERIMENTAL

The UV spectra were measured on a Beckmann Model DU or DB instrument, in 95% ethanol or methanol. IR spectra were taken on Perkin Elmer Infracord at C.D.R.I., Lucknow.

The NMR and mass spectra were recorded at C.D.R.I., Lucknow, University College, Swansea, England, and at Institut fur Pharmaseutische Chemie, Munster, West Germany.

BDH or E.Merck Analar solvents were used for chromatographic purposes. Other solvents were purified by standard methods.

Melting points were taken on a Kofler Block and are uncorrected.

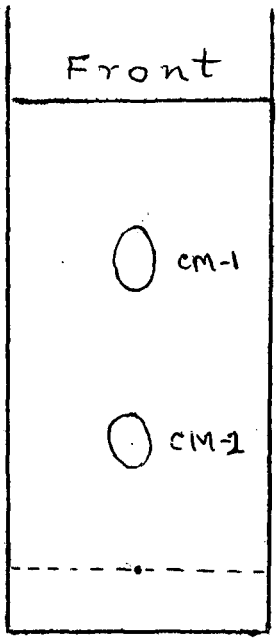
Silica gel for column chromatography was obtained from National Chemical Laboratory, Poona. Alumina was obtained from Woelm, Eschwege, Germany.

Calliasarma macrophylla

Petroleum ether extract

Air dried powdered leaves of Calliasarma macrophylla (10 kg) were exhaustively extracted with petroleum ether (60-80) in a soxhlet for 10 days. The extract was concentrated and kept in a refrigerator when it deposited a yellow green solid (6 gm). The solid was filtered and refluxed several times with petroleum ether to get dissolve out waxy and low melting substances. Repeated crystallisation of the defatted product from petroleum ether-benzene gave white shining needles, which melted however in a range between 125-135°.

TLC of the product on silica gel plate run with benzene-ethyl acetate (75:25) and developed with phosphomolybdic acid, showed it to be a mixture of two components.



25% ethyl acetate-benzene

Column chromatography over silica gel column:

The solid (5 g) was dissolved in the minimum amount of benzene and mixed with silica gel (20 g) so as to give a slurry which was stirred with a rod till most of the benzene had evaporated. The resulting powder was completely freed from benzene under vacuum in a desiccator. It was then placed on a column of silica gel (500 g) prepared with petroleum ether benzene (1:1).

The column was eluted with petrol-benzene (1:1), benzene, benzene-ethyl acetate (5 to 50%) and finally with pure ethyl acetate.

The petrol-benzene (1:1) eluate afforded only waxy and low melting products and was discarded.

Fractions obtained on elution with 5% ethyl acetate-benzene afforded on evaporation of solvent a crystalline solid which after recrystallisation from petroleum had m.p.124°. The product was initially designated as CH-1 but this was changed subsequently to calliterpenone mono acetate.

IR (KBr) 3390 (-OH), 1725 and 1745 (-OCOCH$_3$) 1700 (cyclohexanone) cm^{-1}; NMR ($CDCl_3$, values): 3.79 (2H,s, -CH_2OCOCH_3), 7.88 (3H,s, -OCOCH_3) 8.91, 8.96 and 9.0 (9H,s each,3 C-methyls). In the mass spectrum, major peaks were recorded at m/e 362 (M^+; 4%), 302 (21%), 290 (22%), 289 (100%), 271 (10%), 247 (14%), 230 (14%), 216 (22%), 199 (22%), 159 (25%), 149 (15%), 147 (13%), 137 (12%), 133 (15%), 123 (12%), 121 (15%), 109 (26%), 107 (26%), 105 (22%).

Analysis: Found: C, 73.42%; H, 9.76%; Active H, 0.32%
Calculated for $C_{32}H_{54}O_4$
C, 72.89%; H, 9.45%; Active H, 0.30%.

Fractions obtained on elution with 30% ethyl acetate-benzene, afforded a second product which after crystallisation from benzene melted at 132-33°. This was initially designated as : CH-2 but the name given by A.Chatterjee et al calliterpenone, was subsequently adopted.

$[\alpha]_D^{19}$ + 30 ($CHCl_3$); IR (KBr) 3300-3400 (broad band, OH), 1708 (cyclohexanone) cm^{-1}; NMR ($CDCl_3$, δ values), 6.18 and 6.38 (1H, d, each, J=11 cps, $-CH_2OH$), 8.92, 8.97, and 9.00 (3H, s, each, 3 C-methyls); Mass spectrum: Peaks at 320 (M^+; 4%), 302 (7%), 290 (33%), 289 (100%), 271 (21%).

Analysis: Found: C, 74.91%; H, 10.66%; Active H, 0.24%
Calculated for $C_{20}H_{32}O_3$:
C, 74.96%; H, 10.06%; Active H, 0.23%

Calliterpenone monoacetate:

Calliterpenone (100 g) was left over night with acetic anhydride (1 ml) and pyridine (1 ml). The mixture was poured crushed ice and extracted with ether. The ether extract was washed successively with sodium bicarbonate and water and dried (Na_2SO_4). The residue left on evaporation of ether, crystallised from petroleum ether m.p. 124° yield (80 mg).

Hydrolysis of calliterpenone monoacetate:

Calliterpenone monoacetate (100 mg) was refluxed with 10% methanolic KOH on a water bath for 4 hrs. Most of the solvent was removed under vacuum and the product extracted with ether after dilution with water. The ether extract was washed with water, dil.HCl, water and dried (Na_2SO_4). The residue left on evaporation of ether, crystallised from benzene m.p. 151-53°, mixture m.p. with calliterpenone gave no depression.

Lithium Aluminium hydride reduction of calliterpenone:

Lithium Aluminium hydride (200 mg) was taken in a 100 ml three necked flask and covered over with dry ether (25 ml). A solution of the calliterpenone (200 mg) in dry tetrahydrofuran (25 ml) was added slowly from a dropping funnel under stirring while the ether refluxed gently from the head of the reaction. The addition was complete in 2 hrs.

The stirring was continued for 30 minutes more and the excess of the hydride was then destroyed by careful addition of ether saturated with water and finally of water to the vigorous stirred and cooled reaction mixture. The ether was decanted off, dried (Na_2SO_4) and evaporated. The residue left was crystallised from petroleum ether m.p. 260°, yield 150 mg.

Analysis: Found: C, 73.8%; H, 10.60%;
Calculated for $C_{30}H_{36}O_3$
C, 74.0%; H, 11.18%

Reduction of calliterpenone with hydrazine hydrate:

Calliterpenone (152 mg) hydrazine hydrate (1 ml) potassium hydroxide (0.5 g) and diethylene glycol (10 ml) were heated under reflux for 30 minutes, the solution was cooled and diluted with water. The product on extraction with ether and crystallisation melted at 260°. It was identified as calliterpenone triol by mixed m.p. and co-TLC. Yield (130 mg).

Analysis: Found: C, 73.88%; H, 10.46%
Calculated for $C_{30}H_{36}O_3$
C, 74.0%; H, 11.18%.

Acetylation of calliterpenone triol:

Calliterpenone triol (100 mg) was dissolved in 1 ml pyridine and (1 ml) acetic anhydride and the mixture was left for 30 minutes at 0°. It was then poured over crushed ice and the acetate extracted with ether. The ether was washed with water, bicarbonate, and water and dried (Na_2SO_4). Residue left evaporation of ether crystallised from petroleum ether m.p.180°.

TLC (silica gel, benzene) of this product showed it to be a mixture of three components. The major

constituents of the mixture was eluted from a silica gel column with 50% petroleum ether-benzene. It was crystallised from light petroleum m.p. 198-200°.

IR (KBr) 3420 (-OH); 1730 and 1250 ($-O-\overset{O}{\overset{\|}{C}}-CH_3$) cm^{-1}.

NMR ($CDCl_3$, values): 5.8 (2H, s, CH_2-OAc); 7.94 (3H, s, $O-C-CH_3$) 9.02, 9.2 and 9.3 (3H, s, each 3-C-methyls).

Mass spectrum: Peaks at m/e 364 (M^+; 1%), 292 (20%), 291 (100%), 286 (12%), 273 (52%), 231 (18%), 149 (18%), 147 (16%), 135 (33%), 133 (26%), 123 (21%), 121 (36%), 109 (27%), 107 (26%), 105 (26%).

Analysis: Found: C, 72.49%; H, 10.42%
Calculated for $C_{22}H_{36}O_4$
C, 72.49%; H, 9.96%

Of the other two components, one was found to be the unreacted callitrpenone triol, m.p.260°. The quantity of the third was not sufficient for characterisation.

Periodate oxidation of callitarpenone triol:

Callitarpenone triol (200 mg) was dissolved in (20 ml) of methanol and (140 mg) ($NaIO_4$) dissolved in (2 ml) water were mixed together. The mixture was left at room temperature for 24 hours, diluted with water and the product was extracted with ether. The evaporation of ether gave a crystalline product m.p. 158°, yield (150 mg).

Dehydration of reduced periodate product:

100 mg of this product was taken in 6 ml dry benzene to which 2.5 ml freshly distilled PCl_3 was added. The mixture was refluxed for 2 hours, cooled poured into water and extracted with ether. The ether extract was washed with water, bicarbonate, water and dried (Na_2SO_4). The residue left on evaporation of ether showed three spots on TLC plate.

The mixture was chromatographed over a column of silica gel. On elution with 25% petroleum ether-benzene an oily product (shown by TLC to be a single entity) was obtained (50 mg), but attempted crystallisation from different solvents did not provide any crystalline material.

Lithium Aluminium hydride reduction of the dehydration product:

Solution of the above dehydration product (50 mg) in tetrahydrofuran (10 ml) was reduced as before with lithium aluminium hydride (50 mg) in ether (10 ml). The product obtained after the usual work up was crystallised from petroleum ether m.p. 100-103°. The NMR spectrum of this showed that more than three 3-tertiary methyl signals and protons in the olefinic region integrating for less than 1 proton which suggested that it was a mixture. The amount was insufficient for further purification.

Preparation of callitarpenone acetonide (XCV):

To callitarpenone (500 mg) in dry acetone (100 ml), conc.HCl (10 drops) was added and the mixture was left overnight. The reaction mixture was worked up by concentration, addition of excess water and extraction with ether. The ether extract was washed successively with water, bicarbonate, water and dried (Na_2SO_4). The residue obtained on evaporation of ether crystallised from light petroleum-benzene mixture in fine needles m.p.164-66°.

TLC (silica gel 25% benzene ethyl acetate) revealed it to be a mixture of two compounds with very similar mobility.

Separation was affected by chromatography over neutral Al_2O_3 (Woelm, activity grade II) which afforded the acetonide (major component) from the 5% and the minor component from the 10% benzene ethyl acetate eluates.

The acetonide was crystallised from petroleum ether-benzene m.p.175°, yield (350 mg).

IR (KBr): 1720 (Cyclohexanone) cm^{-1}; NMR ($CDCl_3$, values): 5.95 and 6.1δ (1H, d, each, J=9 cps, CH_2-O-), 8.63 and 8.66 (3H, each, acetonide methyls) 8.93, 9.0, 9.05 (3H,s,each, 3 C-methyls).

Analysis: Found: C, 75.61%; H, 10.16%

Calculated for $C_{23}H_{36}O_3$

C, 76.12%; H, 10.07%.

The second product (minor component) crystallised from petroleum ether in flakes, m.p.135-36°. Yield (50 mg).

Analysis: Found: C, 75.79%; H, 10.68%
Calculated for $C_{23}H_{36}O_2$
C, 76.12%; H, 10.07%.

Periodate oxidation of calliterpenone (C).

Calliterpenone (100 mg) was dissolved in methanol (10 ml) $NaIO_4$ (70 mg) dissolved in water (1 ml) was added to it. The mixture was setaside for 24 hours at room temperature. The product was extracted with ether after dilution with water. The ether extract was washed twice with water and dried (Na_2SO_4). The residue left on evaporation of ether was crystallised from light petroleum-benzene m.p.170-75°.

TLC showed formation of one product and presence of impurities which were removed by chromatography over silica gel.

17-Norphyllocladane-3-16-dione was obtained from the benzene eluate and was crystallised from petroleum ether m.p. 180°, yield (80 mg).

IR (KBr): 1710 and 1750 cm^{-1}; Mass spectrum: peaks at m/e 288 (M^+; 100%), 245 (15%), 233 (32%), 203 (72%), 202 (74%), 190 (38%), 139 (24%), 125 (16%), 107 (21%), 105 (18%), 98 (25%).

Analysis: Found: C, 79.41%; H, 10.31%
Calculated for $C_{19}H_{28}O_2$
C, 79.12%; H, 9.79%.

Wolff-Kishner reduction of callitetrapenone (C).

A mixture of callitetrapenone (500 mg) hydrazine hydrate (85%, 3 ml) digol (25 ml) and potassium hydroxide (1 g) was refluxed on oil bath for 1½ hrs. Water and excess of hydrazine was removed by a take off condenser until the temperature had rised to 195-200. The refluxing was continued for 8 hrs and reaction mixture then cooled diluted with water and the product extracted with ether. The residue left on evaporation of ether, crystallised from petroleum ether, m.p. 165-68°.

TLC (silica gel, 20% benzene-ethyl acetate) showed it to be a mixture of three components which moved very close together.

All the three components were separated by chromatogra hy over silica gel using (benzene-ethyl acetate) gradient elution technique. Fractions of 50 cc each were collected and their composition determined by TLC.

Phyllocladane-16,17-diol (XV) was obtained as the main product (230 mg), crystallised from petroleum ether m.p. 171-72°.

IR (KBr): 3400 (-OH), no carbonyl absorption. Mass spectrum: Peaks at m/e 306 (M^+, 5%), 276 (74%), 275 (100%), 257 (45%), 233 (32%), 137 (63%), 123 (60%), 109 (52%), 107 (30.5%), 95 (30.5%).

Analysis: Found: C, 78.19%; H, 11.50%

Calculated for $C_{20}H_{34}O_2$

C, 78.38%; H, 11.18%.

Of the two products present in the mixture one was the unreacted sailiterpenone (15 mg). The third product after crystallisation from petroleum ether benzene melted at 114-15°. Its IR spectrum shows a prominent OH band at 3400 and C=O at 1725 cm^{-1}. The NMR spectrum showed the CH_2OH proton as singlet at 6.28. Its amount was insufficient for further characterisation.

Periodate oxidation of phyllocladane-16,17-diol (XV), to 17-norphyllocladane-16-one (XX).

To phyllocladane-16,17-diol (100 mg) in methanol (10 ml) was added $NaIO_4$ (70 mg) in water (1 ml) and the reaction mixture kept over night at room temperature. The product was worked up as usual and crystallised from light petroleum m.p.98-100°, (50 mg).

Analysis: Found: C, 82.71%; H, 11.28%
Calculated for $C_{19}H_{30}O$
C, 83.15%; H, 11.02%.

Acetylation of phyllocladane-16,17-diol (XV):

Phyllocladane-16,17-diol (100 mg) was acetylated with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature. The mixture was worked up as usual after 12 hrs and the product was crystallised from petroleum ether m.p.135-40°.

Analysis: Found: C, 75.05%; H, 10.14%
Calculated for $C_{22}H_{36}O_3$
C, 75.81%; H, 10.41%.

Reaction of calliterpenone acetonide (XCV) with NBS in anhydrous CCl_4:

Calliterpenone acetonide (150 mg), NBS (120 mg) CCl_4 (12 ml) were refluxed together for 2 hrs with dibenzoylperoxide (1 mg) on water bath. The solution was then filtered from insoluble material and worked up by adding water and extraction with ether. On evaporation of ether a yellow oil which was shown by TLC to be a mixture with two prominent spots.

Column chromatography over silica gel did not afford any crystalline material.

Reaction of calliterpenone with ethanedithiol/BF_3:

Calliterpenone (340 mg) was dissolved in ethanedithiol (1 ml) and acetic acid (2 ml) and BF_3 in acetic acid (1 ml) added.

A solid started to separate after 15 minutes which was filtered after 1½ hr and washed with methanol colourless crystals m.p.180°. (Yield,170 mg).

Desulphurisation with Raney nickel.

Raney nickel used for catalytic reduction was prepared from "Nickel Aluminium Alloy" (BDH) according to the procedure given in Organic Synthesis Vol. III pp. 176

The above thioketal was boiled for 8 hrs with an excess of Raney nickel in dioxan (200 ml). The solution was then filtered from the catalyst and dioxan removed under vacuum. The product obtained was oily and could not be crystallised. Attempted crystallisation from petroleum ether gave amorphous solid, m.p.90-100.° After chromatography over silica gel it melted at 92-100°.

Bromination of calliterpenone acetonide (XCV):

Preparation of bromine-ether complex: An addition product of bromine and ether was prepared by slowly adding dry ether to 0.1 g of ice cooled bromine until a second layer formed above the dark red insoluble oil. The temperature was not allowed to rise above 20°.

The addition product was used immediately and into a stirred solution of calliterpenone acetonide (100 mg) in dry ether (10 ml) over a period of 15 minutes at room temperature.

The faint yellow solution was washed with bicarbonate and water dried (Na_2SO_4) and the ether was evaporated. The residue was crystallised from petroleum ether benzene gave yellow needles m.p. 190-92°(15%).

Analysis: Found: C, 61.25%; H, 8.00%
Calculated for $C_{23}H_{35}O_3Br$.
C, 62.28%; H, 7.9%.

Baeyer Villiger oxidation of calliterpenone acetonide (XCV):

Perbenzoic acid was prepared according to the procedure given in literature (73). Amount of ingradients are as follows:

$MgSO_4 \cdot 7H_2O$ (250 mg), NaOH (3 g), H_2O_2) (30%,7.5 ml) Benzoyl peroxide (6 g).

Calliterpenone acetonide (600 mg) was dissolved in chloroform (25 ml) and an excess of a chloroform solution of perbenzoic acid was added. TLC of the reaction mixture at intervals showed very slow formation of the lactone. There was very little change in the comparative intensities of the spots of the starting material and the lactone and other spot also began to appears. The reaction was terminated after five days by addition of a few drops of dimethyl sulphide. The residue obtained on evaporation of chloroform was chromatographed over silica gel which brought about a neat separation of the lactone from the unreacted calliterpenone acetonide. Crystallisation from petroleum ether benzene gave a nicely crystalline product m.p.163-65° (50 mg).

Analysis: Found: C, 74.01%; H, 10.95%
Calculated for $C_{23}H_{36}O_4$
C, 73.36%; H, 10.07

SeO_2 oxidation of calliterpenone acetonide (XCV):

Calliterpenone acetonide (100 mg) and selenium dioxide (200 mg) in benzene (20 ml) was refluxed for two hrs on a water bath. The solution was filtered and the filtrate was washed successively with water, bicarbonate, water and dried (Na_2SO_4). The reddish residue obtained on evaporation of benzene indicated the presence of selenium in the reaction product, and could not be crystallised.

Most of the selenium was removed by repeated column chromatography over silica gel and the product obtained was crystallised from benzene-acetone in fine needles m.p. 260 (30 mg).

Analysis: Found: C, 64.5; H, 8.48%
Calculated for $C_{23}H_{34}O_3Se$
C, 63.5%; H, 8.0%.

Callicarpa macrophylla (seeds)

Petroleum ether extract:

Powdered seeds of Callicarpa macrophylla (1 kg) was extracted thrice with petroleum ether. The combined extract was concentrated to a small volume and left in refregerator when it deposited a yellow solid which was filtered and was shown by TLC to be a mixture.

The mixture was acetylated by heating with acetic anhydride/pyridine for 1 hr and allowing to stand over night. The mixture of acetate was resolved by chromatography over silica gel using gradient elution (benzene-ethyl acetate). This afforded three products which the second in order of elution was identical with calliterpenone monoacetate. The amount of the 3rd was insufficient for characterisation. The first product formed the major constituent of the mixture (700 mg). After crystallisation from petroleum ether benzene it had m.p.205-10°, when crystallised from methanol it melted at 268-70°

Analysis: Found: C, 75.06%; H, 10.11%

Calculated for $C_{32}H_{50}O_4$

C, 75.30%; H, 9.73%

literature (66) m.p. of oleanolic acid acetate { from benzene 197° / from methanol 268°

The identity was confirmed by comparative IR.

Callicarpa longifolia (Verbenaceae)

Petroleum ether extract:

Air dried powdered leaves of Callicarpa longifolia (3 kg) were extracted with petroleum ether in a soxhlet for 7 days. The ether extract was concentrated and kept in refrigerator when it deposited a yellow solid (2 g). The solid was filtered and chromatographed over a column of silica gel. The column was eluted with petrol benzene and benzene-ethyl acetate.

The petroleum ether benzene eluate afforded lowing melting product and was discarded.

The benzene-ethyl acetate (5-50%) afforded callilterpenone m.p. 153-54°.

Analysis: Found: C, 74.88%; H, 10.16%
Calculated for $C_{20}H_{32}O_3$
C, 74.96%; H, 10.06%

Flueggea microcarpa (Euphorbiaceae)

Alcohol extract:

The defatted leaves were extracted with ethanol for three days, the extract taken to dryness under reduced pressure, dissolved in water and extracted first with ether and then with ethyl acetate in a liquid-liquid extractor. The ethyl acetate extract was concentrated and the compound was precipitated by gradual addition of petroleum ether. It was then filtered and the residue obtained was dissolved in a small amount of acetone and left in refrigerator. After a few days colourless crystals began to form on the sides of the flask and the crystalline mass gradually increased. The crystals were collected and washed with petroleum ether and crystallised from acetone m.p.135-36°. Recrystallisation from acetone and drying under vacuum for six hours raised the melting point to 236°, (lit.m.p.236-38°)(87). Identified as bergenin.

Analysis: Found: C, 49.83%; H, 5.06%
Calculated for $C_{14}H_{16}O_9 \cdot H_2O$
C, 49.8%; H, 5.1%

Penta-acetyl bergenin:

Bergenin (200 mg) was left over night with acetic anhydride (2 ml) and pyridine (2 ml) at

room temperature. The mixture was poured on crushed ice and extracted with ether. The ether extract was washed successively with dil. H_2SO_4, sodium bicarbonate and water, dried (Na_2SO_4) and ether evaporated. The residue was crystallised from ethanol, m.p. 196-97°.

Analysis: Found: C, 53.3%; H, 4.84%
Calculated for $C_{14}H_{11}O_4(OAc)_3$
C, 53.5%; H, 4.9%

Di-O-methyl bergenin

Bergenin (300 mg) in methanol (10 ml) was treated with ethereal diazomethane and left overnight in a refrigerator. The solid obtained on evaporation of ether was crystallised from water-methanol, m.p. 195°.

Analysis: Found: C, 53.2%; H, 5.9%
Calculated for $C_{16}H_{20}O_9$
C, 53.9%; H, 5.7%.

Acetylation of the methyl ether:

The methyl ether (100 mg) in acetic anhydride (1 ml) and pyridine (1 ml) was left for 24 hrs at room temperature. The usual work up and crystallisation from methanol, m.p. 131-32°.

Rhamnus triqueta (Rhamnaceae)

Alcohol extract

The defatted heartwood was extracted with boiling ethanol in a soxhlet for three days. The extract was taken to dryness under reduced pressure, the residue was dissolved in 500 ml water and washed with petroleum ether and ether in order to get rid of green colouring matter. It was then extracted first with ether and then with ethyl acetate in a liquid-liquid extractor.

Ether extract

The ether extract was reduced to a small volume and kept in a refrigerator for a few days. The deposited solid was filtered and on TLC run in benzene:pyridine:formic acid (36:9:5) showed the presence of 6 components. The major components of the mixture were separated by preparative TLC.

First component was obtained as a yellow crystalline solid (50 mg) m.p.185-86°. It could not be further investigated due to insufficient amount.

The second component was obtained in substantial amount (500 mg), crystallised from $CHCl_3$ in shining flakes, m.p.256°. Identified as emodin.

Analysis: Found: C, 65.20%; H, 3.84%
Calculated for $C_{15}H_{10}O_5$
C, 66.67%; H, 3.70%

Preparation of emodin acetate:

Emodin (100 mg) was left over night with pyridine (1 ml) and acetic anhydride (1 ml). It was then worked up as usual and crystallised from methanol as yellow needles, m.p. 185-90°.

Analysis: Found: C, 62.98%; H, 3.9%
Calculated for $C_{21}H_{16}O_8$
C, 63.90%; H, 4.04%.

Preparation of methyl ether:

Emodin (100 mg) was dissolved in methanol (5 ml) and treated with ethereal diazomethane and left over night at 0°. It was then worked up as usual and crystallised from methanol, m.p.170°.

The third product was obtained in form of gummy solid which could not be crystallised and appeared to be a glycoside. The same product was obtained later on from ethyl acetate extract (confirmed by comparative TLC).

Ethyl acetate extract:

The ethyl acetate extract on concentration and cooling gave a brown amorphous solid. It was filtered and washed several times with ethyl acetate. It was found insoluble in ether, soluble in water and supposed to be a glycoside.

Hydrolysis:

The glycoside (100 mg) was hydrolysed by refluxing with 10% methanolic HCl (25 ml) for two hours on water bath. The solvent was reduced to its half and a little water was added, a thick yellow precipitate was formed which was filtered and crystallised from methanol m.p.253-54°.

Analysis: Found: C, 66.13%; H, 4.04%
Calculated for $C_{13}H_{10}O_5$
C, 66.6%; H, 3.70%

Acetylation of aglycone:

Aglycone (100 mg) heated over water bath for 1½ hr with pyridine (1 ml) and acetic anhydride (1 ml). It was then worked up as usual and crystallised from methanol, m.p. 182-85°.

Analysis: Found: C, 62.98%; H, 3.9%
Calculated for $C_{21}H_{16}O_8$
C, 63.2%; H, 4.04%

Identification of sugar moiety:

The aqueous hydrolysate was evaporated to dryness under reduced pressure, the residue left dissolved in small amount of methanol and applied to the Whatman paper No.1 along with authentic sample of rhamnose, galactose and glucose. The chromatogram was developed with butanol saturated with water and spots were developed with water and spots were developed with aniline phthalate, when presence of rhamnose revealed. Thus the glycoside was found to be rhamnoside of emodin.

Coleobrachia canescitifolia (Labiatae)

Petroleum ether extract:

Air dried powdered leaves of the plant (10 kg) were extracted with petroleum ether in a soxhlet for a week. The petroleum ether extract was concentrated and left in a refrigerator. After few days, a yellow solid began to separate out. It was filtered and crystallised from petroleum ether m.p.125-30°.

TLC of the product on a silica gel plate with benzene-ethyl acetate (4:1) revealed the presence of two distinct spots under a UV lamp.

Separation of the compounds:

The residue (2 g) dissolved in a small amount of benzene was absorbed on a column of silica gel. The column was run first with petroleum ether and then with petroleum ether-benzene, benzene and finally with ethyl acetate.

The petroleum ether and benzene eluates showed no fluorescent spots on TLC and on evaporation gave oily low melting products which were discarded.

The 5% ethyl acetate-benzene eluate afforded a product (I) (500 mg) which crystallised from benzene in fine needles, m.p.165-67°.

UV: 263, 306 nm ; IR (KBr): 1630 cm^{-1}; NMR ($CDCl_3$ values): 6.0, 6.14, (5,6,7-OCH_3); 3.2 and 3.28 (3 and 8-H); 2.5 (3',4',5'-H); 2.1 (2',6'-H).

Analysis: Found: C, 69.55%; H, 5.19%
Calculated for $C_{18}H_{16}O_5$
C, 69.22%; H, 5.16

This compound was identified as 5,6,7-trimethoxy flavone by mixed melting point with an authentic sample kindly provided by Prof.Shiagaki Kosenova.

The 10% ethyl acetate-benzene eluate afforded a second product (II) in quite substantial amount (900 mg). It was crystallised from pure benzene, m.p.140-41°.
UV: 266, 320 nm; IR (KBr): 1640 cm^{-1}; NMR ($CDCl_3$ values): 6.01, 6.07, 6.12 (5,6,7,4'-OCH_3); 3.20 and 3.42 (3 and 8-H); 2.95 and 3.04 (3',5'-H); 2.12 and 2.22 (2',6'-H).

Analysis: Found: C, 67.23%; H, 5.33%
Calculated for $C_{19}H_{18}O_6$
C, 66.66; H, 5.30%

The compound identified as 5,6,7-4'-tetra methoxy flavone by mixed m.p with an authentic sample kindly provided by Prof. Tatum.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. E.Wenkert,
Chemistry and Industry, 282 (1955).

2. R.F.Church and R.E.Ireland,
J.Org.Chem., 28, 17, (1963).

3. R.E.Ireland, P.W.Schiess,
J.Org.Chem., 28,6 (1963)

4. J.D.Connolly, R.McCrindle, K.D.H.Murray and K.H.Overton,
J.Chem.Soc., 274 (1966).

4(a) R.E.Corbett and S.G.Wyllie,
Tetrahedron Letters, 29, 1903 (1964)

4(b) J.D.Connolly, R.McCrindle, K.D.H.Murray and K.H.Overton
Tetrahedron Letters,, 29, 1983 (1964).

5. Quarterly Reviews,
No.1, Vol.XV, (1961) pp.56.

6. B.E.Cross, R.H.B.Galt and J.R.Hanson,
J.Chem.Soc. 2944 (1963).

7. R.McCrindle and K.H.Overton,
Advances in Org.Chem. Methods & Results
Vol.V, Interscience Publishers (1965) pp.86.

8. C.W.Brandt,
Chemical Abstract, 47, 10506g (1953).

9. P.K.Grant and R.Hodges,
Chemical Abstract, 54, 4524g (1960)

10. P.K.Grant and R.Hodges,
Chemical Abstract, 54, 19753g (1960).

11. L.H.Briggs, D.F.Cain, R.C.Cambie and D.R.Davis,
J.Chem.Soc. 1040 (1962).

12. L.H.Briggs, D.F.Cain, D.R.Davis and J.K.Wilmshurst,
Chemical Abstract, 3490g (1960).

13. Carl Djerassi, M.Gaio and I.A.Mitscher,
J.Amm.Chem.Soc., 81,2306 (1959).

14. C.Djerassi, P.Quitt, and K.Mosettig,
J.Am.Chem.Soc., 83, 3720 (1961).

15. C.Djerassi "Optical Rotatory Dispersion",
McGraw Hill Book Co., N.Y. (1960).

16. W.Klyne,
J.Chem.Soc., 3072 (1953).

17. R.B.Turner and K.M.Gaudhirt,
Tetrahedron Letters, 231-3 (1961).

18. L.H.Briggs, B.F.Cain, R.C.Cambie, B.R.Davis,
P.S.Rutledge and J.K.Wilmshurt,
J.Chem.Soc., 1345 (1963).

19. F.Dodler, H.Licht, K.Mosettig and P.Quitt,
J.Am.Chem.Soc., 82, 246 (1960).

20. H.Verbrueggen, C.Djerassi, K.Mosettig, P.Quitt
J.Waters and U.Beglinger,
J.Am.Chem.Soc., 83, 3163 (1961).

21. R.A.Bell, R.E.Ireland, R.A. Partyka,
J.Org.Chem., 3741 (1962).

22. J.W.Apsimon and J.O.Edwards,
Canad.J.Chem., 40, 896 (1962)

23. L.Ruzicka,
Experientia, 10, 357 (1953)

24. See Ref.7, pp.84

25. B.E.Cross and J.C.Stewart,
Tetrahedron Letters, 30, 2193 (1960)

26. G.Nugel, L.Leds, J.M.Heller, D.W.Theobald and C.Ourisson,
Bull.Soc.Chim., France (1974 (1963).

27. P.deMayo,
The Higher Terpenoids, Vol.III, Interscience,N.Y.
(1959) pp.46.

28. J.L.Simonsen and D.H.R.Barton, The Terpenes, Vol.III,
(1952).

29. A.J.Haagen-Smit, Progress in the Chemistry of
Natural Products, Vol.XII, (1955).

30. R.Misra, R.C.Pandey and Sukh Dev,
Tetrahedron Letters,,49, 3731 (1964).

31. W.A.Ayer, E.K.McDonald and J.B.Stothers,
Canad.J.Chem.,41, 1113 (1963).

32. A.W.Burgstahler, H.Ziffer and U.Weiss,
J.Am.Chem.Soc., 83, 4660 (1961).

33. J.A.Pople,
Proc.Roy.Soc. (London) Ser A, 239,550 (1957).

34. P.R.Jefferies, R.S.Rosich and D.E.White,
Tetrahedron Letters, 16, 1793 (1963)

35. E.Wenkert and P.Beak,
Tetrahedron Letters, 358 (1961).

36. A.Gaudemer, J.Polonsky and E.Wenkert,
Bull.Soc.Chim. France, 407 (1964).

37. C.A.Henrick and P.R.Jefferies,
Tetrahedron Letters, 21, 1175 (1965).

38. C.A.Henrick and P.R.Jefferies,
Tetrahedron Letters, 1507 (1964).

39. See Ref.36

40. C.A.Henrick and P.R.Jefferies,
Aust.J.Chem., 17, 915 (1964)

41. F.Piozzi, D.Venturella, A.Bellino and R.Mondelli,
Tetrahedron, 24, 4073 (1968).

42. T.J.King and J.P.Yardley,
J.Chem.Soc., A, 4900 (1961).

43. C.R.Narayanan and N.K.Venkatasubramanum,
Tetrahedron Letters, 41 3639 (1965)

44. N.S.Bhacca and D.H.Williams,
Application of NMR Spectroscopy in Org.Chemistry,
Holden Day, San Francisco (1964) pp 47.

45. W.H.Hui, K.K.Ng, H.Fukamiya, N.Kotsuda and K.Nakanishi,
Phytochemistry, 10, 1617 (1971)

46. G.H.Nadkarni, Indian Materia Medica, pp.835

47

47. Kazuyoshi Kawazu and Tetsuo Mitsui,
Tetrahedron Letters, 18, 2319 (1966)

48. Shigeki Hasegawa, Natsuki Kato and Katsura Munakata,
Phytochemistry, 11, 2363 (1972).

49. Chikao Nishino, Kazuyoshi Kawazu and Tetsuo Mitsui,
Agr.Biol.Chem., 35 (12), 1921-30 (1971).

50. L.F.Fieser and M.Fieser,
Steroids, Asian Publishing House (1960), pp.821.

51. P.S.Rao, G.P.Sachdev, T.R.Seshadri and H.B.Singh,
Tetrahedron Letters, 43,4885 (1968).

52. M.C.Perezamador and F.Garcia Jimenez,
Tetrahedron, 22 (6), 1937,42 (1966).

53. D.A.H.Taylor,
J.Chem.Soc., 1360 (1967).

54. L.F.Fieser and M.Fieser,
Steroids, Reinhold, New York (1959).pp.370

55. Huang Minlon,
J.Am.Chem.Soc., 71 3301 (1949).

56. D.H.R.Barton, D.A.J.Ives and B.R.Thomas,
J.Chem.Soc., 2056 (1955).

57. R.B.Woffett and J.H.Hunter,
J.Am.Chem.Soc., 73, 1973 (1951).

58. A.Chatterjee, S.K.Deamukh and S.Chandrasekaran,
Tetrahedron, 28, 4319 (1972).

58a L.Horner and E H Winkelmann, Newer Methods of Preparative Organic Chemistry, Vol III (1964) pp 183 Reference cited therein

59. A.G.Gonzalez and J.M.Rocha,
Tetrahedron Letters, 7, 599-602 (1969).

60. A.G.Gonzalez, J.L.Breton, B.M.Fraga and J.G.Luis,
Tetrahedron Letters, 33, 3097 (1971).

61. M.R.Kupton and S.J.Nelson
J.Am.Chem.Soc., 640 (1955).

62. E.E.Jones and G.Roberts,
J.Am.Chem.Soc. 3888, (1952).

63. D.H.R.Barton and G.A.Morrison,
Progress in the Chemistry of Natural Products,
Vol.XIX, Wien-Springer-Verlag,(1961)pp 267.

64. N.S.Bhacca and D.H.Williams,
Application of NMR Spectroscopy in Organic Chemistry,
pp. 48, Holden-Day, San Francisco (1964).

65. See Ref.59

66. See Ref.60

67. R.J.Abraham and J.S.E.Holker,
J.Chem.Soc., 806 (1963).

68. E.L.Chicalberti and P.R.Jefferies,
Aust.J.Chem., 440 (1968).

69. See Ref.38

70. K.H.Overton, H.G.Weir and A.Wylie,
Proc.Chem.Soc. 211 (1961).

71. J.R.Hanson and A.F.White
Tetrahedron, 4839 (1970).

72. D.H.R.Barton, J.S.Fawcett and B.R.Thomas,
J.Chem.Soc., 3147 (1951).

73. Y.Ogata and Y. Sawaki,
Tetrahedron, 3332 (1967).

74. David Rosenthal,
J.Org.Chem., 32, 4084 (1967).

75. G.P.Moss and S.A. Nicolaidis,
Chem.Comm. 1077 (1969).

76. G.H.Witham,
J.Org.Chem., 2016 (1960).

77. H.Ishihara and S.Kato,
Tetrahedron Letters, 3751 (1972).

78. R.N.Chopra, S.L.Nayar and I.C.Chopra,
Glossary of Indian Medicinal Plants,
C.S.I.R. New Delhi (1956) pp. 121.

79. See Ref.78, pp.211.

80. J.W.Reynen, G.R.Lopter and A.E.Williams,
J.Phy.Chem., 63, 1061 (1959).

81. See Ref.78, pp.74

82. T.J.Mabry, K.R.Markham and M.B.Thomas,
The Systematic Identification of Flavonoids,
Springer Berlag (1970).

83. J.H.Tatum and R.E.Berry,
Phytochemistry, 11, 228 (1972)

84. J.F.Kutney and J.K.Hall,
Phytochemistry, 10, 3239 (1971)

85. S.Hosozawa, H.Kato and K.Munakata,
Phytochemistry, 11, 2362 (1972)

86. Heilbron Dictionary of Organic Compounds,
Vol.IV (Eyre & Spottiswoode, London) (1953) pp.19

87. Evelyn May and L.J.Haynes,
J.Chem.Soc. 2231 (1958).

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